

**ETHNOBOTANY, PHYTOCHEMISTRY, AND TOXICITY OF *ALOE*
SPECIES FROM EAST AND SOUTH OF THE GREAT RIFT VALLEY IN
ETHIOPIA**



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BIODIVERSITY**

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DEDICATION

I dedicate this dissertation to the late my father-in-law Ato Mersha Adessa Gurcha, whose excited face I recall when I told him I was beginning my PhD. He was very eager to see my success but passed at the middle of my PhD journey.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Dissertation is my authentic manuscript and all sources of materials used for preparation of the PhD Dissertation have been properly acknowledged. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this Dissertation.

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BIOGRAPHICAL SKETCH

The author was born on September 29, 1972 at Gursum town, Eastern Hararge of Oromia Region, Ethiopia. He completed his primary and junior education at Gursum Primary and Secondary School from 1978-1988. He then joined the then Bahir Dar Pedagogical Science College of Addis Ababa University now Bahir Dar University in 1988 and graduated with Diploma in Biology. He was employed by the Ministry of Education and served as Primary and Junior school teacher at Chelenko in the former Webera Awraja and now Meta Wereda from 1990 to 1998. At the end of his service, he joined the undergraduate program in Addis Ababa University and completed BSc degree in Biology in 2001. In the same year (2001), he was transferred to Haramaya secondary and preparatory school and served from 2002-2004. In 2005 he joined the East African Regional Program for Sustainable Use of Dryland Biodiversity known as RPSUD/SIDA-SARIC hosted by Addis Ababa University to study for the Degree of Master of Science in dryland biodiversity conservation and management and obtained his MSc degree in 2006. From July 2006-2011 he worked as Researcher on the Ethiopian local products under the Ethiopian Home Garden Project (EHGP) in the value chain/Social anthropology /ethnobotany/ biodiversity/ecology team employed by Addis Ababa University in the international research project funded by French GEF, IRD/MNHN, Paris. At the end of the project, he joined Haramaya University as a Lecturer in 2011 and was promoted to Assistant Professor in 2014. In 2015, he joined Haramaya University, School of Animal and Range Sciences to pursue his study leading to the Degree of Doctor of Philosophy in range ecology and biodiversity.

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ACRONYMS AND ABBREVIATIONS

AAU	Addis Ababa University
FGD	Focus Group Discussion
HPTLC	High-Performance Thin Layer Chromatography
RFC _s	Relative Frequency of Citation
UV _s	Use Value
[M-H]-	Negative ionization mass spectrometry
AF	Afar Floristic Region
ANOVA	Analysis of Variance
APG	Angiosperm Phylogeny Group
AR	Arsi Floristic Region
BA	Bale Floristic Region
CITES	Convention on International Trade in Endangered Species
CV	Cultural Value Index
DD	Data Deficient
ESI	Electro-Spray Ionization
EU	Environmental Uses
Fd	Food
F _{ic}	Informants' Consensus Factor
HA	Harargae Floristic Region
HPLC-PDA	High Performance Liquid Chromatography-Photodiode Array detector
HU	Haramaya University
IUCN	International Union for the Conservation of Nature
LC	Least Concern
LD	Lethal Oral Dose
m/z	Mass-to-charge ratio
Md	Medicines
Mt	Materials
NMR	Nuclear Magnetic Resonance
NT	Near Threatened

OECD	Organization for Economic Cooperation and Development
P	Probability
PIC	Prior Informed Consent
R _f	Retention factor
R _{is}	Relative Importance Index
RP	Reverse Phase
SD	Sidamo Floristic Region
SEM	Standard Error of Mean
SU	Shewa Floristic Region
SU	Social Uses
TH	Traditional Healers
TLC	Thin Layer Chromatography
TUT	Tshwane University of Technology
UC	Use Categories
UHPLC-MS	Ultra-High Pressure Liquid Chromatography–Mass Spectrometry
UR	Use Reports
UV-DAD	Ultraviolet-Photodiode Array Detection
VP	Vertebrate Poisons
VU	Vulnerable
WHO	World Health Organization

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LIST OF ARTICLES AND MANUSCRIPTS

This thesis is based on the work contained in the following papers:

Paper I. Ethno-medicinal and Bio-cultural Importance of Aloes from South and East of the Great Rift Valley Floristic Regions of Ethiopia (Published on *Heliyon* 2020, 6(6): e04344)

Paper II. Phytochemical isolation and identification of phenolic compounds from leaf exudates of Ethiopian Endemic *Aloe* species using HPTLC technique (Prepared for submission)

Paper III. Phytochemical characterization of leaf exudates of Ethiopian Endemic *Aloe* species using UHPLC-MS-DAD techniques (Submitted to *Phytochemistry* I)

Paper IV. Acute oral toxicity test from leaf exudates of 17 *Aloe* species from East and South of the Great Rift Valley in Ethiopia (Published on *Advanced in Traditional Medicine*, 20(3): 1-12. <https://doi.org/10.1007/s13596-020-00497-9>).

Ethnobotany, Phytochemistry and Toxicity of *Aloe* Species from East and South of the Great Rift Valley in Ethiopia

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ABSTRACT

Interest in the ethnomedicinal and biocultural values, and phytochemical profile of *Aloe* species have been raised over the years, due to its popularity as an ingredient in pharmaceuticals, cosmetic formulations, and food supplements. In view of the broad number of *Aloe* species, 560 species and 21 infraspecific taxa, only few have been cultivated and commercialized at the global level and none from the 46 Ethiopian *Aloe* species with 67.3% endemism. This might be due to the limited knowledge of their valuable bioactive compounds and safety concerns since some species have been reported as potentially toxic. Therefore, ethnobotanical investigation, phytochemical characterization, quantification, and toxicological evaluation were conducted on targeted *Aloe* species of Ethiopia that could allow exploitation of enormous potential of the Genus for emerging cosmetic and pharmaceutical industries in the country. A total of 210 respondents (152 men and 58 women) were involved to provide information in the analysis of ethnomedicinal and bio-cultural values. Interviews, focus group discussion (FGD), and guided field walks were methods used to retrieve the knowledge. Relative frequency of citation (RFC_s), informants' consensus factor (i_cF), use value (UV_s), relative importance index (RI_s), and cultural value index (CV) were analyzed. Non-parametric Kruskal Wallis and Wilcoxon tests were performed using R software. Phytochemical profiles of 17 *Aloe* species were quantified using fully automated high-performance thin layer chromatography (HPTLC) and ultra-high pressure liquid chromatography–mass spectrometry (UHPLC-MS) coupled with ultraviolet-photodiode array detector (UV-PDAD). Data were analyzed using visionCATS and Masslynx (version 4.2) softwares. Oral acute toxicity test from leaf exudates of 17 *Aloe* species were conducted using Swiss albino mice (n=5/cage) following OECD 423 guide. A single oral dose of 300 and 2000mg/kg body weight at a rate of 10 mL/kg administered and major toxicity signs were monitored; and relative body and organs weights were measured on a daily basis for 14 days. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Twenty-three *Aloe* species were recorded in the study areas with 196 use-reports (UR) and 2158 citations, grouped into six major use categories (N_{UC}=6). Medicinal use categories accounted for 149 use-reports (76%) with 1607 citations. *Aloe megalacantha* subsp. *alticola*, *A. trichosantha* subsp. *longiflora* and *A. calidophila* were species with the highest numbers of UR (23, 20 and 18, respectively). Higher proportion of medicinal UR (87%, 75% and 61.1% respectively) was recorded for all the three species than other uses. *Aloe calidophila* has highest values in all indices UV (11.72), RFC (0.68), RI (0.89), and CV (6.2). With regard to use per plant parts, leaf exudate accounted for 111 UR (49.1%) of which 92.9% was used for

medicinal purposes. *Aloe retrospiciens* and *A. ruspoliana* were reported poisonous to carnivores. F_{ic} values of the six major use categories ranged from 0.86 to 0.22. Elderly people (>60) had better knowledge than 25-40 and 41-60 age groups (Kruskal-Wallis chi-squared=12.17, df=3, $p=0.006$) and there was a significant difference in depth of ethnomedicinal knowledge between age groups ($p<.001$). The UHPLC-MS chromatogram of 14 *Aloe* species resulted in 56 peaks corresponds to compounds among which 35 (62.5%) were isolated and characterized. Anthrones and chromones were found dominant compound classes accounting for 23 compounds (65.7%) among the isolated and characterized compounds. The most common compounds were aloin A/B isolated from 11 species, aloinoside A/B from 11 species, and microdontin A/B from eight species. In addition, a number of unique compounds like, 7-O-methylaloesin, trihydroxy octadecenoic acid, 7-hydroxy-8-O-methylaloin B, deacetylitoraloin, 6'-O-coumaroyl aloesin, 7-O-methyl-2'-feruloylaloesin, 1-hexanol-pentosyl hexoside, 1-O-methylplicalaloside, isorabichromone, and caffeoyl ester of aloesin were isolated and characterized from Ethiopian endemic *Aloe* species. The phytochemical characterization of *A. pirottae*, *A. megalacantha* subsp. *alticola*, *A. trichosantha* subsp. *longiflora* were done and resulted in unique characteristic compounds. In acute oral toxicity test, mice groups treated with exudates of *Aloe calidophila*, *A. trichosantha* subsp. *longiflora*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivae*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei* and *A. yavellana* did not show any sign of toxicity at both dose levels. Median Lethal Oral Dose (LD_{50} value) of these groups was estimated to be >2000mg/kg body weight. Groups treated with *A. harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 showed overt signs of toxicity and significance difference ($P<.05$) of percentage change in body weight, relative liver weight, and food consumption compared to control group. *Aloe ruspoliana* and *A. retrospiciens* treatment groups showed severe level of toxicity signs and mortality of mice at both doses, in which LD_{50} value was >300mg/kg body weight. These findings will assist emerging cosmetic and pharmaceutical industries in selecting specific Ethiopian *Aloe* species for resource exploitation through wide-scale farming in marginal and non-agricultural croplands suitable for *Aloe* cultivation.

Keywords: *Aloe* exudate, Anthrones, *A. pirottae*, *A. ruspoliana*, *A. retrospiciens*, chromones, ethnomedicine, toxic *Aloe*.

1. INTRODUCTION

1.1. Background

The genus *Aloe* L. (Family Asphodelaceae and order Asparagales) has 560 accepted species and 21 infraspecific taxa ([The Plant List, 2013](#)). It is renowned for its use in herbal medicine throughout its native range in sub-Sahara Africa, the Arabian Peninsula, Madagascar and the Mascarene Islands ([Grace et al., 2009a](#)). The dried latex extracted from the leaves has been used medicinally in Africa, Asia, Europe and the Middle East for hundreds of years ([Morton, 1961](#); [Reynolds, 1996](#); [Grace et al., 2008](#); [Sebsebe and Nordal, 2010](#); [Bjorå et al., 2015](#)). The many and varied traditional uses recorded for *Aloe* species include the treatment of wounds and skin complaints, malaria, microbial infections, and complaints of the digestive system ([Steenkamp and Stewart, 2007](#); [Jia et al., 2008](#); [Grace, 2011](#); [Belayneh et al., 2012](#); [Anteneh and Negussie, 2014](#); [Radha and Laxmipriya, 2015](#)). In addition, commercial preparations containing *Aloe* species include laxative drugs, health drinks and tonics, after shaving gel, mouthwash and toothpaste, hair tonic and shampoo, and skin-moistening gel ([Oldfield, 2004](#); [WHO, 2003](#); [Reynolds, 2005](#)).

In recent years, the genus *Aloe* gained significant interest among consumers and researchers due to medicinal and cosmetic values of the abundant secondary metabolites extracted from leaves ([Grace et al., 2008](#); [Chen et al., 2012](#)). These increasing interest in the phytochemical profile of *Aloe* species has been appreciated by its popularity as an ingredient in pharmaceuticals, cosmetic formulations, and food supplements ([Javed and Atta-Ur, 2014](#); [Cardarelli et al., 2017](#)). However, despite the broad number of *Aloe* species, only few species have been grown and commercialized at the global level. This might be due to the limited knowledge of their effective bioactive compounds, which have been reported to possess a number of phytochemical compounds of secondary metabolites from leaf and root extracts ([Dagne et al., 2000](#); [Lucini et al., 2015](#); [Cardarelli et al., 2017](#)).

The most popular traditionally used and commercialized product of *Aloe* species was leaf exudate (juice and dried/aloe bitter). The dried aloe leaf exudate commercially known as aloe bitter has been produced by evaporating water using direct sunshine or boiling or freeze drying

to produce a yellow to brown hard and shining crystal/powder material (Steenkamp and Stewart, 2007). The beneficial health-promoting properties present in the exudates of *Aloe* leaves are attributed to their diverse phytochemical composition (Lucini *et al.*, 2015; Cardarelli *et al.*, 2017). The phytochemical studies on limited number of species of the genus *Aloe* indicated that over 200 bioactive compounds belonging to different classes such as anthraquinones, anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes, and flavonoids have so far been reported (Reynolds, 1985; Dagne, 1996; Bisrat *et al.*, 2000; Ombito *et al.*, 2015; Cardarelli *et al.*, 2017). These classes of compounds have been shown to possess antiviral, anti-tumour, and antibacterial activities (Jia *et al.*, 2008; Grace *et al.*, 2009b; Akaberi *et al.*, 2016). Numerous in vitro and in vivo pharmacological studies as well as clinical trials have confirmed the traditional uses of *Aloe* as wound healing, anti-inflammatory, anti-microbial, anti-plasmodial, anti-malarial, antioxidant, anti-ulcer, anti-diabetic, hypoglycaemic, anti-cancer, anti-viral, and anti-hyperlipidemic activities (Grace *et al.*, 2008; Radha and Laxmipriya, 2015; Gelila *et al.*, 2011; Anwar *et al.*, 2014; Biruktawit *et al.*, 2015; Tibebu *et al.*, 2018). It has been postulated that these biological activities of *Aloe* extracts can be ascribed to the synergistic action of several phytochemicals compounds rather than from a single component (Dagne *et al.*, 2000; Lucini *et al.*, 2015).

Many traditional healers and users use *Aloe* species for medicinal and cosmetic purposes with the assumption that *Aloe* products are safe; however, this is not always the case with some *Aloe* species. *Aloe* species have potential toxicity, with side effects occurring at some dose levels when ingested or applied topically (Bottenberg *et al.*, 2007). For example, in Kenya and Somalia it was reported that *A. ruspoliana* is used to kill hyenas by smearing meat with the leaf extract (Newton, 1972; Reynolds, 2004). *Aloe buettneri*, *A. lateritia*, *A. rabaiensis*, *A. secundiflora*, and *A. zebrina* have been documented as ingredients in arrow poisons throughout Africa (Neuwinger, 1996). These species have characteristic smell usually described as that of mice or rats associated with the presence of poisonous alkaloid, for which reason the East African *Aloe ballyi* is known as ‘the rat aloe’ (Reynolds, 1996). In this regard, beyond screening the bioactive compounds, toxicological evaluation of the target species is important for proper application of their products. When the leaves of most species of *Aloe* are cut a more or less copious exudate appears yellow at first but rapidly darkening to brown or in a few species dark red. This exudate contains phenolic

compounds, which can be in most *Aloe* species non-toxic but some are extremely toxic, containing a hemlock like substance (Nash *et al.*, 1992; Sánchez-Machado *et al.*, 2017). Some *Aloe* species contain toxic alkaloids (Dring *et al.*, 1984) and certain species, including *A. vera*, may cause adverse reactions (Steenkamp and Stewart, 2007). There are published reports of human deaths resulted from the use of *Aloe* leaves (Neuwinger, 1996; Drummond *et al.*, 1975; Newton, 2001), which could be an overdose use of extracts for medicinal uses. There are also some expectedly poisonous *Aloe* species not studied so far, which were reported as poisonous by the local communities. The leaf juice of *Aloe* species was found from time to time in Africa arrow poison (Reynolds, 1985). Mostly it was not known which species have been used except the following species are certain: *Aloe lateritia*, *A. rabaiensis* and *A. secundiflora* (Neuwinger, 1996). Somali hunters on the Wabi Shebelle in Ethiopia used the leaf of *Aloe* species in meat to poison animals (Neuwinger, 1996). Therefore, beyond their wider uses, toxicological evaluation of *Aloe* species is important for proper application of their products. Especially, the recent widespread exposure of the society to *Aloe* products in Ethiopia, it has been raised safety concerns and its potential toxic activities should be studied.

Ethiopia, a likely centre of diversity for *Aloe*, has 46 identified and documented species with three subspecies, of which 67.3% are endemic. Several of these are highly threatened (Edwards, *et al.*, 1997; Fikre, 2006; Sebsebe *et al.*, 2001; Bachman *et al.*, 2020). In Ethiopia many species of *Aloe* are declining in their natural habitat and require conservation in the wild. Six species of *Aloe* in Ethiopia were classified on the IUCN Red List (IUCN, 2015) as endangered (*A. harlana* and *A. yavellana*), near threatened (*A. tewoldei* and *A. pubescens*), and vulnerable (*A. retrospiciens* and *A. rugosifolia*) and the rest of *Aloe* species remain data-deficient (Weber *et al.*, 2013; Weber and Sebsebe, 2013; Weber, 2013a, 2013b). Despite conservation concerns, aloes have been recognized for their economic potential in Ethiopia (Sebsebe and Nordal, 2010), particularly for livelihood security, economic development and enhancing biodiversity conservation on marginal lands (Mukonyi, 2007; Mukonyi *et al.*, 2007). For example, *Aloe debrana* leaf mesophyll is used in a thickening agent (Sisay *et al.*, 2013) and for treating sisal fibre for packing Ethiopian export coffee (e.g. www.gseventiplc.com). In Borena of the Oromia region, Southeast Ethiopia, the local community has been cultivating *A. calidophila* to collect aloe leaf exudate and gel for small-scale aloe-soap manufacturing (Teshome, 2014; Pers.

observation). In view of the broad number of *Aloe* species in Ethiopia, it is surprising that none of these species (except *A. calidophila*) have been cultivated and commercialized; this might be due to the limited knowledge of the phytochemical potential across these species.

Some research works done in Ethiopia also indicate that the local communities make use of them in traditional medicine to treat major health problems for example, *Aloe gilbertii* for malaria and wound (Fikre, 2013), *A. harlana* for tropical ulcer (Anteneh and Negussie, 2014), *A. pirottae* for malaria (Anteneh et al., 2012), *Aloe sinana* as a wound-healing agent, insecticide, treatment of snake-bite and malaria (Genet et al., 2014). Following such indigenous knowledge related to the bio-cultural uses, some of the endemic *Aloe* species (like, *A. harlana*, *A. trichosantha*, *A. gilbertii*, and *A. citrina*) were investigated for their bioactive phytochemical compounds and antioxidant properties. The existing reports on the isolation, characterization, and efficacy evaluation of bioactive phytochemical compounds from some of the Ethiopian *Aloe* species validated the traditional uses of aloes as well as the presence of new compounds (Gelila et al., 2011; Anwar et al., 2014; Yitagesu et al., 2014; Biruktawit et al., 2015). However, there is no commercial production of the gel and exudates for natural products from Ethiopian aloes while the country has the potential that could supply local to regional markets in east Africa.

In this sense, the isolation, characterization, and quantification of bioactive compounds in *Aloe* species could allow the exploitation of enormous potential locked up in the Ethiopian *Aloe* species for the cosmetic, pharmaceutical, and additives in food industries. Though comprehensive works are still lacking on the phytochemistry of Ethiopian aloes, the limited works done so far on some Ethiopian *Aloe* species indicated that they possess diverse chemical constituents as well as unique types of compounds (Dagne et al., 2000; Anwar et al., 2014; Mekdes et al., 2015). Despite the limited reports on the uses and conservation concerns for aloes of Ethiopia, their ethnomedicinal, biocultural values, phytochemical profiles and toxic effects have not previously been assessed enough systematically. It is expected that people will be motivated to conserve resources that are most important to them, in contrast to resources perceived as less useful (Byg and Balslev, 2001, Garibaldi and Turner, 2004). In this regard, further effort to improve the perception of local community towards the resource is needed. This can be achieved by indicating the invisible but potential values of the genus *Aloe* for effective

conservation of the genus *Aloe*. In addition, it has been hypothesized that the local communities in all the study areas make use of *Aloe* species arbitrarily for similar purpose and that there was no variation between the different cultural/ethnic communities on the use of *Aloe* species. Moreover, as a result of the huge medicinal demand for *Aloe* species as well as the morphological similarities among species, there is a high possibility that the species are often (erroneously) substituted with each other for different medicinal uses (Grace *et al.*, 2009b).

Therefore, this PhD dissertation research comprehensively investigates the ethnomedicinal values, biocultural importance, and emic perception of the wild population status of *Aloe* species in the East and South of the Great Rift Valley floristic region of Ethiopia. Moreover, using advanced techniques of HPTLC, UHPLC-MS and HPLC-PDA, isolation and characterization of compounds from the leaf exudates of targeted *Aloe* species have been analysed for identification of the potential Ethiopian *Aloe* species in industrial relevance and maximize their conservation. Similarly, due to an increasing interest in the phytochemical and pharmacological potentials of *Aloe* species in Ethiopia, the need for toxicity tests of this valuable natural resource cannot be under-emphasized. Therefore, the last chapter of this study has been focused on the acute oral toxicity test for 17 prioritized *Aloe* species to generate basic scientific information that provide safety information for their existing diverse traditional medicinal applications, product development and promotion, utilization under cultivation and wild conservation.

1.2. Research Questions

1. How the local communities are using *Aloes* and where the economic potential lies?
2. What are the phytochemical constituents of endemic and indigenous *Aloe* species targeted in this study?
3. Are there any exceptional uses related to antioxidant properties in targeted *Aloe* species?
4. What are the antioxidant properties of the phytochemical compounds in targeted *Aloe* species?
5. How do we relate the phytochemical constituents with that of the claimed therapeutic and cosmetic values of these *Aloe* species?
6. Are there poisonous species of *Aloe* in the study areas?
7. How are the toxicity effects of each extracts of the target *Aloe* species?

1.3. Hypothesis

- The local communities in all the study areas make use of aloes indiscriminately for same purpose.
- There is strong relationship between the bio-cultural uses and bioactive compounds found in the target *Aloe* species.
- All endemic/indigenous *Aloe* species have exceptional local uses related to antioxidant properties if used as food purposes.
- All aloe species used by the local communities are non-toxic.

1.4. Objectives of the study

1.4.1. General objective

- This PhD research project is aimed to conduct a comprehensive investigation on the bio-cultural multipurpose uses, identify the top priority species, and isolation and characterization of bioactive compounds with analysis of their antioxidant properties of top priority species for proper correlation between long lasted traditional uses with that of the bioactive compounds. In addition, or safely reason in their application at any level, toxicity tests of the leaf exudates/latex/sap will be evaluated for the top priority species, which are promising for commercial application and to use this neglected resource as an opportunity for livelihood support and degraded ecosystem rehabilitation.

1.4.2. Specific objectives

- To investigate the multipurpose traditional (Bio-cultural) values comprehensively and analyze the claimed local applications of *Aloe* species in the study areas.
- To isolate, identify, and characterize the phytochemical constituents of the leaf exudates that can provide a scientific rationale to support the claimed local application of targeted *Aloe* species.
- To evaluate the toxicity from leaf exudates of targeted *Aloe* species (prioritized *Aloe* species) of the study areas.

2. LITERATURE REVIEW

2.1. The Genus *Aloe* L.

2.1.1. Phylogenetic and Morphological Descriptions

The genus *Aloe* L. includes monocots with succulent leaves that belongs to the Asphodelaceae family in the order Asparagales (James *et al.*, 2016). The family Asphodelaceae is further divided into three subfamilies named as Asphodeloideae, Hemerocallidoideae and Xanthorrhoeoideae. The genus *Aloe* belongs to Asphodeloideae. After Linnaeus, Berger revised *Aloe* classification in 1908, which received wide acceptance. Following Berger, the South African botanist, G.W. Reynolds greatly contributed to the knowledge of this genus through the publication of two large, well illustrated books, one in 1950 covering the southern African *Aloe* species and the second in 1966 covering the tropical African and the Madagascar species (Edwards *et al.*, 1997; Sebsebe and Nordal, 2010).

The taxonomy of *Aloe* at the species and infraspecific levels is unstable and resulted in some confusion (Cock, 2015). This is primarily attributed to the fact that, *Aloe* species display uneven patterns of variation among populations and species. The morphology of leaves, flowers and inflorescence varies widely and inconsistently (Smith and van Wyk, 1991). Recently, several new classification reviews have been adopted to solve the problems on the position of *Aloe* and allied genera. Besides gross morphology, other characters have been employed by taxonomists to reveal the relationships of the genera in the subfamily Asphodeloideae. Some of these characters are pollen (Steyn *et al.*, 1998); phytochemistry (Viljoen and van Wyk, 1996; Viljoen *et al.*, 2001), isoenzyme (Van der Bank *et al.*, 1995), and molecular phylogeny (Grace *et al.*, 2013; Daru *et al.*, 2013; Manning *et al.*, 2014).

In the past decades, the position of *Aloe* and its allied genera in the family and subfamily had been changing several times. For example, in the near past, it has been assigned to the family Aloaceae (now included in the Asphodeloidae) or to a broadly circumscribed family Liliaceae (the lily family). The APG IV System (Byng *et al.*, 2016) places the genus *Aloe* in the family Asphodelaceae, subfamily Asphodeloidae. Within the subfamily, it was placed in the tribe Aloeae or Aloooideae. Four core genera were recognized in the subfamily until recently: *Aloe* L.

(ca. 400 species) the largest genus in Aloioideae, followed by *Haworthia* Duval (ca. 70 species), *Gasteria* Duval (23 species), and *Astroloba* Uitewaai (seven species) (Klopper *et al.*, 2010). Molecular phylogenetic studies, particularly from 2010 onwards, indicated that the genus *Aloe* does not form a monophyletic group. There is agreement among many researchers that Asphodeloideae can be further divided into two monophyletic groups: a clade comprising *Aloe* and its immediate relatives, the 'aloids', and a clade of 'asphodeloid' genera (Grace *et al.*, 2013; Manning *et al.*, 2014). Daru *et al.* (2013) examined the evolution of morphological characterstates used in early taxonomic work in Aloioideae and most were demonstrated to be homoplasious.

Manning *et al.* (2014) produced a phylogeny in which *Aloe* was divided into six genera: *Aloidendron*, *Kumara*, *Aloiampelos*, *Aloe*, *Aristaloe*, and *Gonialoe*. Most recently, as of October 2017, Aloee (or Aloioideae) comprises eleven genera: *Aloe* L. (>500 species), *Aloiampelos* Klopper & Gideon F. Sm. (seven species), *Aloidendron* (A. Berger) Klopper & Gideon F. Sm. (six species), *Aristaloe* Boatwr. & J.C. Manning (one species), *Astroloba* Uitewaai. (seven species), *Gasteria* Duval (23 species), *Gonialoe* (Baker) Boatwr. & J.C. Manning (three species), *Haworthia* Duval (23 species), *Haworthiopsis* G.D.Rowley (18 species), *Kumara* Medik (two species), and *Tulista* Raf. (four species) (Grace *et al.*, 2013; Manning *et al.*, 2014; Byng *et al.*, 2016). Among these eleven genera known in Aloioideae, *Aloe* is traditionally by far the most diverse genus including more than 500 species. Although there is general similarity in vegetative morphology among species within the smaller genera, the large genus *Aloe* displays great diversity in this regard. More critically, *Aloe* was only negatively diagnosable, lacking any synapomorphies by which it could be recognized (Daru *et al.*, 2013; Manning *et al.*, 2014). Therefore, the genus *Aloe* with the allied genera in Aloioideae is still more complex which require further taxonomic evidence and molecular phylogenetic analysis.

The species in the genus *Aloe* are all leaf-succulent plants and mostly associated with arid and semi-arid environments infrequently influenced by drought (Grace *et al.*, 2015) many occur in more mesic habitats and several species occur at higher altitude like, *Aloe ankoberensis*, *A. steudneri* and *A. pulcherrima* in Ethiopia and *Aloe polyphylla* in Lesotho. Plants in the genus *Aloe* are recognized by their fleshy, strongly cuticularized rosette leaves usually with spiny

margins. The rosettes are situated on the ground or on stems up to 2 meters. In rare cases, the thick leaves are spaced along a stem rather than crowded in a rosette. The leaves of individual species vary greatly in size, colour and the presence and distribution of prickles on the leaf margins and faces. Though the leaf margin is usually armed with sharp teeth, there are, however, some exceptions, including taxa with almost entire leaf margins, like *Aloe pulcherrima*. All *Aloe* species are evergreen, bisexual and flowers are produced in racemose inflorescences. The inflorescence is usually branched (occasionally simple), the lower branches sometimes branching again. The racemes may be dense or sparse in different species. They are usually erect and round, but in some cases, they are oblique or more or less horizontal in one direction, becoming secund (e.g. *A. secundiflora* Engl.). Each flower is supported by a bract, the shape and size of which are important for the identification of the species (Reynolds, 1996; Edwards *et al.*, 1997). Flowers of almost all *Aloe* species are tubular and brightly coloured red, pink, and yellow and sometimes white and greenish (Reynolds, 1996), unscented, produce abundant nectar (Human and Nicolson, 2008), bird or insect-pollinated flowers, mostly with exserted anthers and style, and flattened, wind-dispersed seeds (Manning *et al.*, 2014). These features point to ornithophily as the pollination syndrome, in which nectarivores (nectar feeding) birds, more of sunbirds, are frequent visitors to *Aloe* flowers. Fruit is a loculicidal capsule. The capsule wall is either papery or slightly woody when mature. The seeds are irregularly 3-sided to flattened, narrowly to broadly winged. The seed coat is covered with an arillus, the colour of which varies from black and grey to brown (Reynolds, 1996; Sebsebe *et al.*, 2003).

Some *Aloe* species are found as dense populations (e.g. *Aloe tewoldei* Gilbert & Sebsebe), whilst others occur as scattered individuals or solitary rosettes (e.g. *A. rivae* Baker). The species in *Aloe* are acaulescent (multi-stemmed) or caulescent (single stemmed) that display a wide range of habits with drastic variation in height (Figure 1) ranging between small perennial herb about 30 cm high (like *A. macrocarpa*, *A. dorotheae* A. Berger), shrubs about 2 m high (like, *A. megalacantha* subsp. *megalacantha*), climbers (like, *Aloe*, subsect. *Prolongatae* A. Berger ser. *Macrifoliae* Haw.), scandent (scramblers such as *A. tenuior* Haw. and *A. ciliaris* Haw.), and large tree up to 18 meters (e.g. *Aloidendron barberae* T. Dyer) tall (Reynolds, 1996; Smith and van Wyk, 1991).



Figure 1. *Aloidendrone barberae* (South Africa), *A. megalacantha* subsp. *megalacantha* (Ethiopia, Dengego mt of Eact Hararge), and *A. macrocarpa* (Ethiopia, Hakim Gara of Harar town).

The leaves are the most commonly used medicinal parts of *Aloe* species, which are heterogeneous and can be divided into three major parts:

- (i) the outer green epidermis, primarily consisting of structural components;
- (ii) the outer pulp region below the epidermis, consisting of vascular bundles of aloitic cells that secrete and store the bitter exudate; and
- (iii) the inner leaf mesophyll, consisting of aloe gel and containing parenchyma cells (Figure 2).

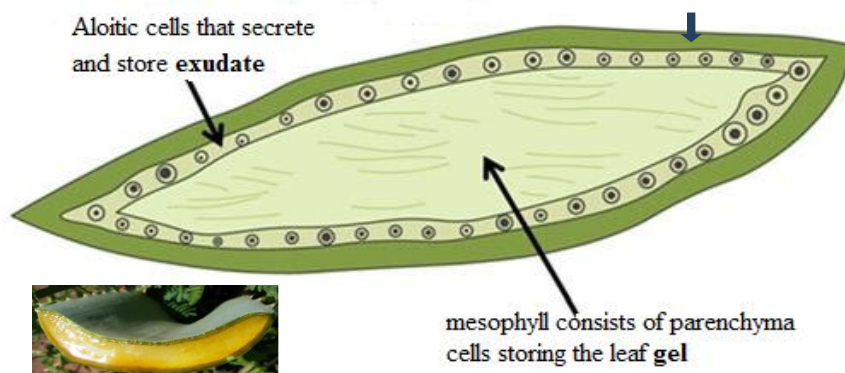


Figure 2. A cross section illustration of *Aloe* leaf covered with epidermis consists of multiple layers with chloroplasts.

Most aloes are xerophytes with structural and physiological adaptations for survival in arid and semi-arid regions. The major structural adaptation is leaf-succulence, having thick and fleshy

leaves with gel, enlarged to accommodate the aqueous tissue (Figure 3a). The leaf cuticle is thick and covered with a layer of wax as well as the outer stomatal pores are sunken, i.e. situated at the base of a suprastomatal cavity. *Aloe* species have groups of cells associated with the vascular bundles, variously called aloitic cells that secrete a mixture of compounds with medicinal value, with the composition varying in different species. This appears as an exudate, usually yellow (Figure 3b) when leaves are broken or cut. The term "Aloe" is also referring to the dried exudate (Figure 3c) extracted from the leaves/aloitic cells ([Sebsebe and Nordal, 2010](#)).



Figure 3. The "gel" from colourless spongy mesophyll (a), yellow exudate (b) and aloe bitter/powder (c) from aloitic cells (Photo courtesy Anteneh Belayneh, 2018).

2.1.2. *Aloe* Distribution and Diversity

Though Aloes are iconic in the African flora, some species are also found in the Arabian Peninsula and few are known from western Indian Ocean islands like, Mauritius, Reunion, Comoros, etc. ([Grace et al., 2009b](#)). The genus is found almost throughout the African continent south of the Sahara Desert, except for the moist lowland forest zones and the western end of West Africa. The majority of species occur in Southern Africa, on the eastern side of the continent, and on Madagascar, in tropical and subtropical latitudes. The Arabian species have clear relationships with the species of northeast Africa. Madagascan species appear not to be closely related to those of mainland Africa and so active speciation seems to have occurred since the separation of these two landmasses. [Reynolds \(2004\)](#), has been classified the distribution of

Aloe in five regions, i.e. southern Africa, Madagascar and Indian Ocean islands, eastern Africa, central and western Africa and Arabian Peninsula (Figure 4).

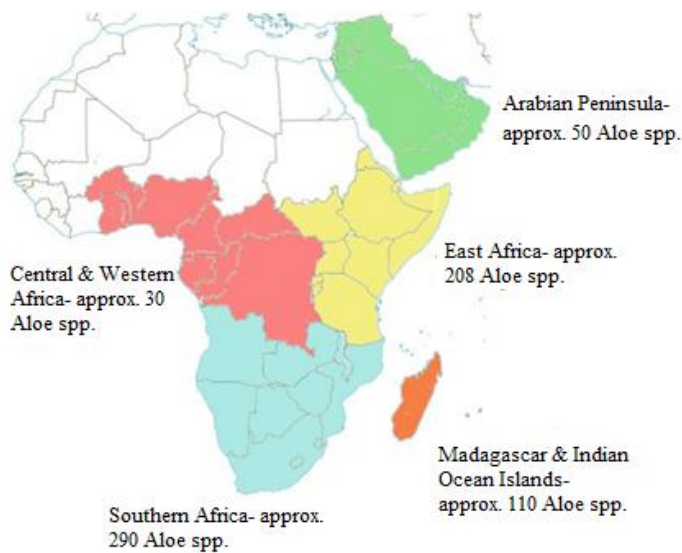


Figure 4. *Aloe* species distribution by region, based on the number of species per country reported summary. (Mascola *et al.*, 2004; Sebsebe *et al.*, 2011; Bachman *et al.*, 2020)

The genus *Aloe* L. (Asphodelaceae) has about 560 accepted species and 21 infraspecific taxa synonyms and unresolved species, subspecies, varieties, and hybrids (The Plant List, 2013). Tough and spiked leaves with unpalatable juice protect them from grazing, gleaming flowers that have an excessive nectar production attract pollinators. The winged seeds aid them for wind dispersal were major adaptation contributed for their diversification. Some species are widespread (e.g. *A. buettneri*, *A. myriacantha*), while most are habitat specialists with narrow ranges and extraordinary rates of endemism, from an estimated 100% in Madagascar, 67.3% in Ethiopia (Anteneh *et al.*, 2020), and 70% in southern Africa (Grace, 2009c). South Africa has the highest number of *Aloe* diversity followed with Madagascar, Kenya, and Ethiopia (Table 1).

Table 1. Priority countries of *Aloe* species distribution, countries, number of species, endemism with percentage

Countries	Number of species	Endemism
South Africa**	170 (species+ infraspecific)	71 (41.8%)
Madagascar	110	110 (100%)
Kenya*	63	50 %
Ethiopia	49 (species+ infraspecific)	33 (67.3%)
Tanzania	44	15 (34.1%)

Mozambique	41	
Somalia	39	29 (75%)
Zimbabwe	38	10 (26.3%)
Yemen	33	18 (54.5%)
Namibia	31	12 (38.7%)
Malawi	27	5 (18.5%)
Angola	27	15 (55.5%)
Swaziland	26	3 (11.5%)
Zambia	22	4 (18.1%)
Saudi Arabia	21	10 (47.6%)
Comoros	17	17 (100%)
Uganda	16	2 (12.5%)
Sudan	12	3 (25%)
Lesotho	10	2 (20%)

* In Kenya species like *A. secundiflora*, *A. turkanensis* Christian, *A. rivae* Baker, *A. calidophila* Reynolds, and *A. scabrifolia* Lavranos and L.E. Newton are reported to be commercially exploited for aloe bitter exudate (Lubia *et al.*, 2008; Mukoyon *et al.*, 2001; Wabuye and Kyalo, 2008).

** Two commercialized *Aloe* species are native to South Africa: *A. arborescens* and *A. ferox*, (e.g. Salehi *et al.*, 2018).

Most of the *Aloe* species are highly threatened at the global level particularly in the Horn of Africa (Backman *et al.*, 2020). Therefore, the genus *Aloe*, with the sole exception of the cultivated species *Aloe vera*, is of conservation concern due to habitat loss and being extensively collected from the wild for horticulture and natural products (Grace *et al.*, 2009a; Backman *et al.*, 2020). Aloes are protected by Convention on International Trade in Endangered Species of Wild Fauna and Flora/CITES (CITES Appendix II, 2017).

2.2. The Traditional/Bio-cultural Values of *Aloe*

The history of traditional uses of the genus *Aloe* dates back almost 6000 years (Morton, 1961). So far, at least one-third of the genus *Aloe* were documented as having some utilitarian values among the diverse cultural communities in all its range of distribution (Grace *et al.*, 2009a). Old literatures stated that aloes have been used therapeutically and for multiple bio-cultural utility for topical and internal effects, certainly since Roman times and perhaps long before (Morton, 1961). It has been stated also, Aristotle persuaded the king Alexander to conquer Socotra Island, located off the east coast of Africa, in order to acquire aloe for use in healing

wounds of soldiers ([Sanchez-Machado et al., 2017](#)). *Aloe* has multiple pharmacological effects on healing wounds, burns and frostbite, constipation, insomnia, stomach disease, pain, haemorrhoids, itching, dermatitis, headache, hair loss, gum disease, kidney disease, blisters, sunburn, chapping, hair loss, genital ulcers, haemorrhoids, boils, mouth irritation and inflammation and more ([Shelton, 1991](#)). In addition, the genus *Aloe* is also included in the WHO list of most used medicinal plants ([WHO, 2001](#)).

The genus *Aloe* L. had recorded history of mentioned and given high rank as an all-purpose herbal plant. It was renowned for its medicinal and cosmetic properties that have been exploited over millennia ([Morton, 1961](#); [Reynolds, 1996](#); [Grace et al., 2009a](#), [Bjørå et al., 2015](#)). Recent literatures extensively reported, the traditional values as anti-oxidant, antimicrobial, anti-inflammatory, anticancer and anti-malarial activities have been reported ([Grace et al., 2008](#); [Chen et al., 2012](#)). For example, *A. ferox*, a source of natural products known as Cape aloes, has been reported to have 158 use records, followed by *A. arborescens* Mill. with 47 use records, *A. perryi* Baker with 53 use-reports, and *A. marlothii* A. Berger with 45 use-reports ([Grace et al., 2009b](#)). However, the most common traditional use was in the traditional health care system with other agents to treat both internal and external human and livestock ailments.

Three distinct preparation of the aloe plants are used in traditional medicine: aloe latex/exudates (commercialized as aloe bitter); aloe gel; and, whole leaf extract. Numerous in vitro and in vivo pharmacological studies as well as clinical trials using aloe latex/exudates have been confirmed the traditional uses of *Aloe* including wound healing, protection against skin damage from x-rays, lung cancer, intestinal problems, anti-ulcer, anti-diabetic, hypoglycaemic, anti-cancer, anti-bacterial, anti-viral, and anti-hyperlipidemic activities ([Grace et al., 2015](#); [Radha and Laxmipriya, 2015](#); [Lizelle et al., 2017](#)). Aloe gel is used for skin ailments such as wound healing, psoriasis, herpes and internally by oral administration in diabetic and hyperlipidaemic patients and to heal gastric ulcers. Whole leaf extract is potentially useful for cancer and improve immune system ([Mascola et al., 2004](#)). Some species of *Aloe* have been used in a wide range of skin and hair care products, and form the basis of health drinks and tonics ([Grace et al., 2009b](#); [WHO, 2003, 2007](#)).

Most of the *Aloe* species were reported to have multiple uses but more of medicinal. Out of the total 1,467 use records for 173 accepted species and infraspecific taxa of *Aloe*, which were collated from the literatures and through personal observation, the medicinal uses were described by 74% of the use records (Grace *et al.*, 2009b). For example, a total of 57 unique uses were mentioned for *Aloe secundiflora* in Kenya, of which about 70 % were for medicinal uses in humans and livestock (Bjorå *et al.*, 2015). The multipurpose uses reported among the Kenyan aloes were for cosmetics, medicinal (human, livestock, and poultry), soil conservation, local brew, anti-termite, poisonous, ornamental, fodder, etc. (Bjorå *et al.*, 2015). For example, *A. lateritia* was more frequently cited for cosmetic use, and *Aloe secundiflora* and *Aloe ngongensis* were often mentioned as fodder plants, during drought, when other fodder plants are scarce. These tips of evidence from the bulk of literature data signify the multiple bio-cultural uses of the genus *Aloe* dominated by ethno-medicinal uses.

Indeed, *Aloe* species have been reported for multiple biological properties, including antibacterial and antimicrobial, antitumor, anti-inflammatory, anti-arthritic, anti-rheumatoid, anticancer, and antidiabetic activities, detoxification, treating constipation, flushing out toxins and wastes from the body, promoting digestion (on gastrointestinal disorders treatment), and reducing illnesses likelihood, as well as for immune system deficiencies (Boudreau *et al.*, 2013; Radha and Laxmipriya, 2015). These multiple bio-cultural uses could signify the existence of diverse and complex phytochemical constituents in wide array of species in the genus *Aloe*. It has been postulated that these biological properties of *Aloe* extracts can be ascribed to the synergistic action of several phytochemicals compounds (Romani *et al.*, 2008; Lucini *et al.*, 2015). That is why, aloes have been provided a fascinating subject for research from a taxonomic, ethnomedicinal, phytochemical, pharmaceutical, horticultural, ecotouristic, and economic points of view. A recent search for “aloe” on Google.com returned more than 225 million results indicated the popularity of the genus in research and utility. However only few species such as *A. arborescens*, *A. ferox*, *A. perryi* and *A. vera* were dominated research and utility at the global level (Salehi *et al.*, 2018).

2.3. Phytochemistry of *Aloe*

Aloes have a number of bioactive compounds that contribute profound values for humans. The genus *Aloe* has reported to possess a number of phytochemical compounds of secondary metabolites from leaf and root extracts. These diverse phytochemicals from limited number of *Aloe* species like, *A. arborescens* and *A. ferox* were known used by food processing, cosmetics, and pharmaceutical industries in many countries (Oldfield, 2004; Grace *et al.*, 2009a; Demissew and Nordal, 2010). It is the leaf exudate which contains diverse phenolic compounds, which can be distinguished chromatographically. Most of the phenolic compounds of exudates were identified as chromones, anthraquinones or anthrones derivatives (Reynolds, 1985). Currently the number of aloe compounds reached to more than 200 belonging to different compound classes (Table 2) such as, anthraquinones, anthrones, chromones, coumarins, pyrones, alkaloids, glycoproteins, naphthalenes, steroids, volatile oils and flavonoids have so far been reported (Reynolds, 1985; Dagne, 1996; Dagne *et al.*, 2000; Viljoen *et al.*, 2001; Cock, 2015; Cardarelli *et al.*, 2017; Salehi *et al.*, 2018). In addition, the nutrients and phytochemicals that have been identified in *Aloe* species include vitamins, minerals, enzymes, simple and complex polysaccharides, fatty acids, indoles, alkanes, pyrimidines, aldehydes, dicarboxylic acids, ketones, phenolic compounds, phytosterols, and alkaloids with potential biological and toxicological activities (Boudreau and Beland, 2006; Boudreau *et al.*, 2013; El Sayed *et al.*, 2016; Salehi *et al.*, 2018).

Table 2. The phytochemical classes and identified phytochemical compounds in the genus *Aloe* (Reynolds, 1985; Dagne, 1996; Dagne *et al.*, 2000; Viljoen *et al.*, 2001; Cock, 2015; Cardarelli *et al.*, 2017, Salehi, *et al.*, 2018)

No.	Phytochemical Class	Compounds
1	Anthraquinones	Aloe-emodin, aloesaponarin, chrysophanol, desoxyerythrolaccin, 1,5-dihydroxy-3-hydroxy methyl anthraquinone, helminthosporin, 7-hydroxyaloe emodin, isoxanthorin, laccaic acid-D-methyl ester, nataloe emodin and its ester nataloe emodin-8-methyl ester, aloechrysone, aloesaponol, asphodelin, bianthracene, elgonicardine, O-glycosides: aloe emodin-11-O-rhamnoside, nataloe emodin-2-O-glucoside, aloesaponol-6-O-glucoside, aloesaponol-8-O-glucoside, aloesaponol-O-methyl-4-O-glucoside, elgonicardine
2	Anthrones	Aloin A and B (collectively called aloin and also often referred to as barbaloin), 5-hydroxyaloin A, 7-hydroxyaloin, 10-hydroxyaloin B, 5-hydroxyaloin A 6'-O-acetate, 7-hydroxyaloin-6'-O-monoacetate, 10-hydroxyaloin-6-O-acetate, homonataloin, nataloin, aloinoside, barbendol, aloe-emodinanthrone,

		chrysophanolanthrone, aloe emodin-10-C-rhamnoside, 6'-O-cinnamoyl-8-O-methyl-7-hydroxyaloin, , homonataloside, littoraloside, microdantin, 8-O-methyl-7-hydroxyaloin, 6'-O-p-coumaroyl-7-hydroxyaloin, 7-hydroxyaloin-4', 6'-O-diacetate, 6'-O-cinnamoyl-5-hydroxyaloin A, microstigmin A, deacetylittoraloin, littoraloin
3	Chromones	Aloesin A-F, iso-aloesin A & D, 7-O-methylaloesin, 7-O-methylaloesinol, 7-O-methylaloesin A, 8-[C-B-D-[2-O-(E)-cinnamoyl]glucopyranosyl]-2-[(R)- 2-hydroxypropyl]-7-methoxy-5-methylchromone, 8-C-glycosyl-7-O-methylaloesinol, C-Glycosyl-7-O-methyl-S-aloesol, 2-acetonil-7- hydroxy-8-(2-furanonyl)-7-hydroxy-5-methylchromone, 7-Hydroxy-2, 5-dimethylchromone, 20-p-O-methylcoumaroylaloesin
4	Coumarins	Feralolide and dihydroisocoumarin glycoside
5	Pyrans and pyrones	Pyran:Bisbenzopyran; Pyron: aloenin, aloenin aglycone, aloenin acetal, aloenin B, aloe-2''-p-O-coumaroyl ester
6	Alkaloids	N-methyltryamine, O,N-dimethyltryamine, γ -coniceine, coniine
7	Benzene, naphthalene and furan derivatives	Protocatechuic acid, methyl-p-coumarate, pluridone, isoeleutherol, isoeleutherol-5-O-glucoside, feroxidin, feroxidin A, Plicataloside, feroxidin B, plicataloside, 5-OH-3-methylnaphto[2,3-c]furan-4(1H)-one, 3-methylnaphto[2,3-c]furan-4(9H)-one, 3-methylnaphto[2,3-c] furan-4,9-dione
8	Flavonoids	Naringenin, dihydroisorhamnetin, apigenin, isovitexin, isorhamnetin, daidzenin, genistein
9	Sterols	Cholesterol, campesterol, b-sitosterol, lupeol
10	Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galacto glucoarabinomannan, pectic substance, xylan, cellulose, acemannan, maloyl glucans, manose, glucose, fructose, galactose, L-rhamnose, aldopentose
11	Enzymes	Alkaline phosphatase, amylase, sscarboxy- peptidase, cyclooxygenase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate, carboxylase, superoxide dismutase
12	Inorganic compounds	Calcium, chlorine, chromium, copper, iron, magnesium, manganese potassium, phosphorous, sodium, zinc
13	Proteins	Lectins, lectin-like substance
14	Vitamins	B1, B2, B6, B12, C, E, b-carotene, choline, folic acid,a-tocopherol
15	Miscellaneous	Arachidonic acid,g-linolenic acid, triglicerides, triterpenoid, gibberillin, lignins, potassium sorbate, salicylic acid, uric acid

Among phenolic compounds identified from *Aloe* species, aloin was widely studied as one of the main biologically active components found at higher concentration in most *Aloe* exudates ([Liao et al., 2006](#); [Javed and Atta-Ur, 2014](#); [Sánchez-Machado et al., 2017a](#)) whereas much lower concentration in the gel ([Cardarelli et al., 2017](#)). While aloes are used as laxative drugs and health drinks, the main purgative principle being aloin (anthrone), which occurs at levels of between 18 and 30% of the dried product (United States Pharmacopoeia, 1995). Unlike aloin the common compound in most *Aloe* species, plicataloside was reported as unique compound in

some *Aloe* species like, *A. chabaudii*, *A. palmiformis*, *A. plicatilis* (southern Africa) and *A. otalensis* and *A. rugosifolia* (restricted to East Africa) (Viljoen *et al.*, 1999).

The source of these compounds is basically the leaf gel (leaf mesophyll) and leaf and root exudates. There are basic compositional variation in phytochemical constituents and concentration levels between the gel and exudate. Unlike the exudate more than 95% of the gel is water followed with polysaccharide. Aloe gel has exhibited various pharmacological activities and a number of pre-clinical and clinical studies (Grace *et al.*, 2008; Lucini *et al.*, 2015) have reported therapeutic effects such as anti-inflammatory, antimicrobial, antiplasmodial, antimalarial, antioxidant and anticancer activities. It has been postulated that these biological properties of *Aloe* gel extracts can be ascribed to the synergistic action of several phytochemicals and antioxidants (Romani *et al.*, 2008; Lucini *et al.*, 2015). Little is known, however, of the relationship between polysaccharide composition and therapeutic value of the leaf mesophyll or aloe gel. Indeed, it is not yet clear whether polysaccharide composition in *Aloe* and related genera should be an important criterion for the selection of suitable taxa for new industries, as little is known of whether leaf mesophyll constituents differ between *Aloe* species, nor whether the market dominant *A. vera* is superior to other species (Grace *et al.*, 2013). Due to the lack of comparative data describing polysaccharides in *Aloe*, it has not been possible to prioritize taxa of interest for their leaf mesophyll using a chemosystematic approach usually applied to specialized (secondary) metabolites, whereby the properties known in one species could be expected to occur in closely related species or within clades on a phylogenetic tree (Harborne, 2000). Likewise, species of *Aloe* used commercially are rather distantly related, and do not conform to a discernible taxonomic pattern (Grace *et al.*, 2013). The local traditional practices of *Aloe* uses focused more on exudates than use of gel in which the greater number of use reports in the different countries by the diverse communities were for medicinal applications.

The most popular traditionally used and commercialized product of aloe species was exudate (juice and dried/aloe bitter). The dried aloe exudate commercially known as aloe bitter has been produced by evaporating water using direct sunshine or boiling or by freeze drying to produce a brown to black hard and shining crystal/powder material (Steenkamp and Stewart, 2007). This exudate contains phenolic compounds of divers classes in which the principal compounds aloin,

aloinoside A, and aloinoside B, aloesin, plicataloside, isovitexin, aloe emodi, aloresin A, and aloenin (Bisrat *et al.*, 2000; Chen *et al.*, 2012; Grace *et al.*, 2013; Lucini *et al.*, 2013; Cardarelli *et al.*, 2017; Salehi *et al.*, 2018). The beneficial health-promoting properties present in the sap or exudates of *Aloe* leaves are attributed to their phytochemical composition and in particular to characteristic polyphenols, which are one of the major groups of compounds acting as free radicals terminators or primary antioxidants (Miliauskas *et al.*, 2004). For example, among eighteen *Aloe* species studied, *A. marlothii* and *A. melanacantha* were found to be rich in total phenolics, flavonoids, and flavonols suggesting that leaf exudates of these specific species could be used as raw material for cosmetic, medicinal and pharmaceutical purposes (Cardarelli *et al.*, 2017). Concentrated preparations of the leaf exudate (“drug aloes”) are taken for laxative effects attributed to anthraquinones (Steenkamp and Stewart, 2007).

The three parts of *Aloe* leaves (Figure 2) have variation in the phytochemical constituents and concentration levels. For example, the outer green mesophyll has been reported to contain anthraquinones, pre-anthraquinones, and their corresponding glycosides (Reynolds, 2004), while the outer pulp region below the epidermis where the bitter exudate is derived predominantly consists of phenolic compounds, including anthraquinones and pre-anthraquinones, anthrones, chromones, coumarins, flavonoids, and pyrones (Dagne *et al.*, 2000).

One of the most widely known constituents of aloe exudate is aloin or barbaloin, which is found in nature as a mixture of two diastereoisomers, aloin A (10R) and aloin B (10S) (Reynolds, 1985; Liao *et al.*, 2006; Javed and Atta-Ur, 2014), commonly known for its laxative effect. The amount of aloin is also extremely variable among different species and highly depends on the growing conditions (Wamer *et al.*, 2003). Even with this variability, this compound has been reported to represent up to 30% of the dry weight of aloe latex/exudate (Rebecca *et al.*, 2003). Aloin is an anthrone-C-glycoside, generally contained in the smelly *Aloe* leaf yellow exudates (Fanali *et al.*, 2010). In vivo studies have demonstrated antioxidant, antimicrobial, and anti-cancer properties of anthraquinones such as aloin (Park *et al.*, 2009; Chen *et al.*, 2012; Pellizzoni *et al.*, 2012). Barbaloin (also named aloin A) was reported for its histamine release inhibitory, anti-inflammatory, antiviral, antimicrobial, anticancer, antioxidant and cathartic effects (Patel *et al.*, 2012).

2.4. Toxicity from *Aloe* Species

Herbal medicines are assumed to be safe due to their long usage in the treatment of diseases according to knowledge accumulated over centuries. However, despite the growing popularity and presumed safety of herbal medicines, adverse effects have become a major safety issue for natural products (Han *et al.*, 2016). Generally, medicinal plants contain bioactive compounds which demonstrate both intra- and inter-species variation in type and content. Plants by virtue of their chemical constituents are potentially toxic; thus, some plants used in traditional medicine are intrinsically toxic. However, recent scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic, and carcinogenic (Schimmer *et al.*, 1994; Higashimoto *et al.*, 1993; Kassie *et al.*, 1996; De Sã Ferrira and Ferrão Vargas, 1999). Consequently, in response to public health concerns, it is important to validate the safety of traditional herbal medicines before their use. It is expected that toxicity studies concerning herbal medicine should reflect their traditional uses to allow for rational discussions regarding their safety for the beneficial uses. Moreover, experimental data on the toxicity profile of medicinal plants and their extracts should be obtained to increase confidence in their safety for human use and in the development of pharmaceuticals (Yuet Ping *et al.*, 2013).

A number of *Aloe* species are in uses as traditional medicines, dietary supplement in a variety of foods, and ingredients in cosmetic products. The literature includes references to several species of edible aloes, their uses including snack foods, famine foods, as a cooked vegetable and an ingredient in preserves (Reynolds, 1996; Grace *et al.*, 2009a, Bjorå *et al.*, 2015). It should be noted that not all species of *Aloe* are edible: some contain toxic alkaloids (Dring *et al.*, 1984) and certain species, including *A. vera*, may cause adverse reactions (Steenkamp and Stewart, 2007). Especially the widespread human exposure to *Aloe vera* products has been raised safety concerns and its potential toxic and carcinogenic activities have been well studied (Guo and Mei, 2016). It has been reported that *Aloe* species have potential toxicity, with side effects occurring at some dose levels when ingested or applied topically (Bottenberg *et al.*, 2007).

Few aloes were reported consisted of toxic exudates. For example, extracts from *Aloe ruspoliana* gave positive test with Dragendorff's reagent indicating the presence of alkaloids with potential

toxicity (Cock, 2015) but not exactly reported as toxic. *A. ruspoliana* is a non-spotted and stemless Aloe with unpleasant smell found in Sidamo, Bale, and Hararge Floristic regions of Ethiopia (Edwards *et al.*, 1997). In Kenya and Somalia, it was reported that *A. ruspoliana* is used to kill hyenas by smearing meat with the leaf extract (Reynolds, 2004). Quite a few number of *Aloe* species were reported as poisonous, because of the presence of the hemlock alkaloid γ -coniciene (oral LD₅₀ = 12 mg/kg for mice) in exudate of leaves (Nash *et al.*, 1992). Out of 224 *Aloe* species (Kew collection) examined by chromatographic methods, 48 species were found to possess alkaloids, in which six were contained piperidine derivatives of coniine, γ -coniceine and conhydrine, which has potential toxicity in oral application. For example, a leaf sap of *A. buettneri* is said to be used as an ingredient of arrow poison in Mali (Nash *et al.*, 1992) though this is not a species known as being poisonous. *A. buettneri* ('na nine') is a fishing poison of the Dogon in Mali. The leaves are used alone or combined with those of *Sansevieria* species (*pulo va*) and the branches of *Euphorbia kamerunica* (*dulu*) (Neuwinger, 1996). These species have characteristic smell usually described as that of mice or rats, for which reason the East African *Aloe ballyi* is known as 'the rat aloe'. There are published reports of human deaths resulted from the use of aloe leaves (Drummond *et al.*, 1975; Neuwinger, 1996; Newton, 2001), which could be an overdose use of extracts for medicinal uses. There are also some expectedly poisonous *Aloe* species not studied so far, which were reported as poisonous by the local communities. In Africa, the leaf exudates of few *Aloe* species were found from time to time for arrow poison used for carnivores hunting. Mostly it was not known which species have been used except the following species are certain: *Aloe lateritia*, *A. rabaiensis*, and *A. secundiflora* (Neuwinger, 1996). Somali hunters on the Wabi Shebelle in Ethiopia used the leaf of *Aloe* species in meat bait to poison animals (Neuwinger, 1996).

In view of the potential toxicity of alkaloids, it has been pointed out that it is important to screen for alkaloids prior to recommending use of an *Aloe* species as medicine (Dagne *et al.*, 2000). Due to the increased popularity and utilization, in-depth studies are needed to investigate the adverse effects, conventional drug interactions, and the possible toxic and carcinogenic effects of *Aloe* preparations and their products (Guo and Mei, 2016). That is why, beyond screening the bioactive compounds, toxicological evaluation of *Aloe* species is important for proper application of their products.

2.5. The Ethiopian Aloes

2.5.1. Diversity

In Ethiopia 46 *Aloe* species were identified and documented with three subspecies (Table 3), among which 39 species and the three infraspecific taxa were endemic and near endemic (nine species), accounting for 85.7%, to the country (Edwards, *et al.*, 1997; Sebsebe *et al.*, 2001; Sebsebe and Nordal, 2010). Near endemic species are having restricted distribution in one or few neighbouring countries. In this case, *Aloe* species shared with only one neighbouring country were considered as near endemic. Moreover, some *Aloe* species are narrowly endemic with restricted distribution in Ethiopia like, *Aloe adigratana*, *A. ankoberensis*, *A. debrana*, *A. ellkeriana*, *A. harlana*, *A. yavellana* (Sebsebe *et al.*, 2011).

Table 3. Ethiopian *Aloe* species and subspecies, endemism, floristic regions, and phenology (Edwards *et al.*, 1997; Sebsebe *et al.*, 2001; Sebsebe *et al.*, 2003; Sebsebe and Nordal, 2010; Sebsebe *et al.*, 2011)

No.	The Ethiopian <i>Aloe</i> species	Endemicity	Leaf spotting	Stem	Floristic region	Flowering period
1	<i>Aloe adigratana</i> Reynolds	Endemic	Spotted	Stem	Tigray	January to April
2	<i>Aloe ankoberensis</i> Gilbert & Sebsebe	Endemic	Non-spotted	Stemless	Shewa	October to February
3	<i>Aloe benishangulana</i> Sebsebe & Tesfaye	Endemic	Non-spotted	Stemless	Welega	April and August
4	<i>Aloe bertemariae</i> Sebsebe & Dioli	Endemic	Spotted	Stemless	Harerge	November to December
5	<i>Aloe calidophila</i> Reynolds	Near endemic	Non-spotted	Stem	Sidamo	September to December, but sometimes in April to May
6	<i>Aloe camperi</i> Schweinfurth	Endemic	Spotted	Stem	Tigray and Welo	March to May
7	<i>Aloe citrina</i> Carter & Brandham	Near endemic	Prominently spotted	Stemless	Sidamo	September to December and from May to June
8	<i>Aloe clarkei</i> L. Newton	Endemic	Scattered spots	Stem	Kefa	Unknown
9	<i>Aloe debrana</i> Christian	Endemic	Non-spotted	Stemless	Shewa, Gojam and Welo	December to February
10	<i>Aloe elegans</i> Todaro	Endemic	Non-spotted	Stemless	Tigray, Welo, Gojam and Shewa	September to December
11	<i>Aloe elkerriana</i> Dioli & McCoy	Endemic	Spotted	Stem	Harerge	unknown
12	<i>Aloe ellenbeckii</i> Berger	Indigenous	Spotted	Stemless	Sidamo	October to November
13	<i>Aloe eumassawana</i> Carter, Gilbert & Sebsebe	Near endemic	Non-spotted	Stemless	Red Sea coast of Eritrea	
14	<i>Aloe friisii</i> Sebsebe & Gilbert	Endemic	Spotted	Stem	Gamo Gofa	January to February
15	<i>Aloe ghibensis</i> Sebsebe & Friis	Endemic	Non-spotted	Stem	Kefa	November and between March and April
16	<i>Aloe gilbertii</i> subsp. <i>gilbertii</i> Reynolds ex Sebsebe & Brandham	Endemic	Non-spotted	Stem	Shewa	October to December

17	<i>Aloe gilbertii</i> subsp. <i>megalacanthoides</i> Gilbert & Sebsebe	Endemic	Non-spotted	Stem	Gamo Gofa	October to November sometimes April to May
18	<i>Aloe harlana</i> Reynolds	Endemic	Non-spotted	Stemless	Harerge	April to May, sometimes September to October
19	<i>Aloe jacksonii</i> Reynolds	Endemic	Spotted	Small stem	Bale	September to October; also in June
20	<i>Aloe kefaensis</i> Gilbert & Sebsebe	Endemic	Spotted	Stem	Kefa	September to November
21	<i>Aloe lateritia</i> Engler	Near endemic	Spotted	Stemless	Sidamo	September to November
22	<i>Aloe macrocarpa</i> Todaro	Indigenous	Spotted	Stemless	Shewa, Hararge, Bale, Arsi, Shewa	October to April
23	<i>Aloe mcloughlinii</i> Christian	Near endemic	Spotted	Stemless	Harerge	April to May
24	<i>Aloe megalacantha</i> Baker subsp. <i>alticola</i>	Endemic	Non-spotted	Stem	Harerge	August to October; sometimes April to May
25	<i>Aloe megalacantha</i> Baker subsp. <i>megalacantha</i>	Indigenous	Non-spotted	Stem	Bale and Harerge	August to October, sometimes also in April
26	<i>Aloe monticola</i> Reynolds	Endemic	Non-spotted	Stemless	Tigray and Welo	February to April
27	<i>Aloe otallensis</i> Baker	Endemic	Non-spotted	Stemless	Gamo Gofa and Sidamo	September to December
28	<i>Aloe parvidens</i> Gilbert & Sebsebe	Near endemic	Spotted	Stemless	Sidamo, Bale, and Harerge	April to May, occasionally September to October
29	<i>Aloe percrassa</i> Todaro	Endemic	Non-spotted	Stemless	Tigray and Gonder	September to October
30	<i>Aloe pirottae</i> Berger	Near endemic	Spotted	Stemless	Sidamo, Bale, and Harerge	November to January
31	<i>Aloe pubescens</i> Reynolds	Endemic	Non-spotted	Stemless	Harerge	February to May
32	<i>Aloe pulcherrima</i> Gilbert & Sebsebe	Endemic	Non-spotted	Stemless	Gonder, Gojam, Welo, and Shewa	July to September
33	<i>Aloe retrospiciens</i> Reynolds & Bally	Near endemic	Non-spotted	Stem	Afar, Bale, and Harerge	March to April, occasionally also from September to December

34	<i>Aloe riva</i> e Baker	Near endemic	Non-spotted	Stemless	Sidamo	September to December
35	<i>Aloe rugosifolia</i> Gilbert & Sebsebe	Indigenous	Spotted	Stemless	Sidamo	July to September
36	<i>Aloe ruspoliana</i> Baker	Indigenous	Non-spotted	Stemless	Sidamo, Bale, Harerge	September to December
37	<i>Aloe schelpei</i> Reynolds	Endemic	Spotted	Stem	Shewa	October to March
38	<i>Aloe schoelleri</i> Schweinfurth	Indigenous	Non-spotted	Stemless	Eritrea	
39	<i>Aloe secundiflora</i> Engler	Near endemic	Non-spotted	Stemless	Gamo Gofa and Sidamo	April to May, sometimes also in August to December
40	<i>Aloe sinana</i> Reynolds	Endemic	Spotted	Stem	Welo and Shewa	December to April
41	<i>Aloe steudneri</i> Schweinfurth	Endemic	Non-spotted	Stemless	Tigray, Gonder and Gojam	August to October
42	<i>Aloe tewoldei</i> Gilbert & Sebsebe	Endemic	Spotted	Stem	Harerge and Bale	October
43	<i>Aloe trichosantha</i> Berger subsp. <i>longiflora</i> Gilbert & Sebsebe	Endemic	juveniles spotted	Stemless	Harerge	almost throughout the year, with records August to May
44	<i>Aloe trichosantha</i> Berger subsp. <i>trichosantha</i>	Indigenous	Non-spotted	Stemless	Tigray	February to May
45	<i>Aloe trigonantha</i> Leach	Endemic	Non-spotted	Stemless	Gonder and Gojam	September to January
46	<i>Aloe vituensis</i> Baker	Near endemic	Spotted	Stem	Sidamo	March to April
47	<i>Aloe welmelensis</i> Sebsebe & Nordal	Endemic	Non-spotted	Stem	Bale	December
48	<i>Aloe weloensis</i> Sebsebe	Endemic	Spotted	Stem	Welo	September to November
49	<i>Aloe yavellana</i> Reynolds	Endemic	Non-spotted	Stem	Sidamo	September to October

The Ethiopian *Aloe* species has been increased from 38 to 46 due to the identification and description of eight new endemic species where the number increased to over 20% in just 12 years (Dioli and McCoy, 2007; Sebsebe *et al.*, 2011). The last four endemic *Aloe* species identified and added to the Flora of Ethiopia and Eritrea were *Aloe benishangulana* Sebsebe & Tesfaye from near Assosa, Benishangul-Gumuz in Welega floristic region; *A. ghibensis* Sebsebe & Friis from the Ghibe Gorge, Kefa floristic region; *A. weloensis* Sebsebe from near Dessie in Welo floristic region and *A. welmelensis* Sebsebe & Nordal along the Welmel River in Bale floristic region (Sebsebe *et al.*, 2011). This has been indicated that still some more unidentified species are expected to exist in the wild.

There are three local centres of endemism recognised in Ethiopia, each with characteristic endemic species (Table 4) (Sebsebe *et al.*, 2001; Sebsebe and Nordal, 2010), that accounted 67.3% of the Ethiopian aloe species.

Table 4. The three local centres of *Aloe* endemism recognised in Ethiopia

Center of endemism	Floristic regions	Number of endemic species	List of endemic species and infraspecific taxa
Northern and central highlands, north and west of the great rift valley	SU, KF, IL, WG, GJ, GD, WU, TU	15	<i>Aloe adigratana</i> , <i>A. ankoberensis</i> , <i>A. benishangulana</i> , <i>A. camperi</i> , <i>A. clarkei</i> , <i>A. debrana</i> , <i>A. elegans</i> , <i>A. monticola</i> , <i>A. percrassa</i> , <i>A. pulcherrima</i> , <i>A. schelpei</i> , <i>A. sinana</i> , <i>A. steudneri</i> , <i>A. trigonantha</i> , and <i>A. weloensis</i>
Eastern highlands and lowlands	AF, HA	7	<i>Aloe bertemariae</i> , <i>A. harlana</i> , <i>A. mcloughlinii</i> , <i>A. megalacantha</i> subsp. <i>alticola</i> , <i>A. pirottae</i> ; <i>A. pubescens</i> ; and <i>A. trichosantha</i> subsp. <i>longiflora</i>
Southern highlands, lowlands and rift valley	AR, BA, SD, GG	11	<i>Aloe elkerriana</i> , <i>A. friisii</i> , <i>A. ghibensis</i> , <i>A. gilbertii</i> subsp. <i>gilbertii</i> , <i>A. gilbertii</i> subsp. <i>megalacanthoides</i> , <i>A. jacksonii</i> , <i>A. kefaensis</i> , <i>A. otallensis</i> , <i>A. tewoldei</i> , <i>A. welmelensis</i> , and <i>A. yavellana</i>

SU = Shewa; KF = Kefa; IL = Ilubabur; WG = Welega; GJ = Gojam; GD = Gonder; WU = Welo; TU = Tigray; AF = Afar; HA = Harargae; AR = Arsi; BA = Bale; SD = Sidamo; GG = Gamo Gofa

2.5.2. Phytogeography of Ethiopian *Aloe*

In Ethiopia *Aloe* species are found in the different natural vegetation types ranging from desert to the Afroalpine vegetations, from below 400 m to 3400 m asl with annual rainfall between 400 and 1500 mm. As a succulent and drought tolerant genus, most of the Ethiopian *Aloe* species are predominantly found in arid and semi-arid environments. The most common arid and semi-arid Aloes like, *Aloe bertemariae* and *A. citrina* are found in the desert and semi-desert scrubland

vegetation type along with highly drought tolerant plant species of small trees and shrubby species of *Acacia*, *Boswellia*, *Commiphora* and succulent species of *Euphorbia* (Sebsebe and Nordal, 2010) in Afar and Hararge floristic regions below 400 m asl. Some *Aloe* species such as *A. calidophila*, *A. ellenbeckii*, *A. gilbertii*, *A. friisii*, *A. retrospiciens*, *A. mcloughlinii*, *A. pirottae*, and *A. otallensis* are found in the *Acacia-Commiphora* woodland and bushland proper in the eastern, central and southern floristic regions of Ethiopia, between 400 and 1800(-1900) m asl. Two of the widespread species *Aloe trichosantha* subsp. *longiflora* and *Aloe gilbertii* subsp. *gilbertii* are found in *Acacia* wooded grassland of the Rift Valley, around the major rift valley lakes between 1300 to 1800 m asl. (Edwards *et al.*, 1997; Fikre, 2013). *A. yavellana* is found inside remnants of *Juniperus* forest, on rocky slopes and in more disturbed areas at altitudes between 1500 m and 2400 m and with an annual rainfall of between 400 and 700 mm (Sebsebe and Nordal, 2010). *A. megalacantha*, *A. rivae*, and *A. secundiflora* are found with *Acacia* bushland wooded grassland at altitudinal range between 1350-2000 m.a.s.l. None of these *Aloe* species were escaped to north and west of the Great Rift Valley floristic regions of Ethiopia. The phytogeographic limitation of these species and other to the east and south of the Great Rift Valley was not documented so far. Some of these endemic species from East and south of the Great Rift Valley Floristic Regions are like, *A. harlana*, *A. pirottae*, and *A. mcloughlinii*. *Aloe benishangulana* and *A. ghibensis* represent a new group that occur in *Combretum-Terminalia* dominated deciduous woodland and wooded grassland, with regular fires, of western Kefa, Ilubabor and Welega floristic regions to the Sudan and the Omo basin between the 400 and 1800 m. One of these species is adapted to regular fires in its natural habitats by growing on rocky outcrops and the other occurs on steep slopes in a river gorge, over which grass-fires would probably pass quickly (Sebsebe *et al.*, 2011). Their phytogeographic distribution were restricted to west of the Great Rift Valley floristic regions of Ethiopia.

The Ethiopian *Aloe* species found in the highland vegetation types is *A. debrana* in *Juniperus-Podocarpus* Afromontane forests in Shewa floristic regions at altitudes between 1500 and 2700 m asl. with annual rainfall between 700 and 1100 mm. *A. ankoberensis* is found in Afroalpine vegetation types in Shewa floristic regions above 3200 m (Sebsebe and Nordal, 2010). Others like, *A. adigratana*, *A. percrassa*, *A. pulcherrima*, *A. monticola*, *A. steudneri*, *A. trigonantha*, and *Aloe weloensis* are found in dry Afromontane forest of the Ethiopian highlands, on the plateaux

of Tigray, Gojam, Gonder, and Wello floristic regions at altitudes between (1600-) 2200 and 3200 (-3300) meters a.s.l. with annual rainfall between 500 and 1500 mm. *A. elegans* and *A. camperi* occur in Afromontane woodland, wooded grassland and grassland. Similarly, none of these species were escaped to the east and south of the Great Rift Valley floristic regions of Ethiopia.

Comparing the two phytogeographic zones of *Aloe* species divided by the Great Rift Valley, the highlands and lowlands of the east and south are inhabited by a number of endemic and indigenous *Aloe* species where their population in the natural habitat is highly threatened (Sebsebe *et al.*, 2000; Fikre, 2006; Anteneh *et al.*, 2012; Anteneh and Negussie, 2015; IUCN, 2015). The IUCN Red List (IUCN, 2015) recorded six species of Ethiopian *Aloe* under 'endangered/EN' (*A. harlana* and *A. yavellana*), 'near threatened/NT' (*A. tewoldei* and *A. pubescens*), and 'Vulnerable/VU' (*A. retrospiciens* and *A. rugosifolia*) categories, in which all are from the aforementioned floristic regions. The rest are under Least Concern (LC) and Data Deficient (DD) categories (Weber *et al.*, 2013a, Weber, 2013b, 2013c; Weber and Sebsebe, 2013).

2.6. Bio-cultural Values of *Aloe* in Ethiopia

The genus *Aloe* had recorded history of mentioned and given high rank as an all-purpose herbal plant. Similarly, in Ethiopia, the local communities make use of aloes in traditional medicine and other bio-cultural uses. The limited works done on the bio-cultural values of Ethiopian aloes indicate that the local communities make use of them for traditional medicines to treat major health problems like, *A. pirottae* for malaria (Anteneh *et al.*, 2012), *A. harlana* for tropical ulcer (Anteneh and Negussie, 2014), *Aloe sinana* as a wound-healing agent, insecticide, treatment of snake-bite, and malaria (Genet *et al.*, 2014). For example, *Aloe* latex/exudates locally called *sibrii* in eastern Ethiopia is used for its laxative effect in traditional health care system and bio-cultural uses as a colon cleanse on the eve of the month of Ramedan by the Muslim community, elderly people (Anteneh and Negussie, 2014). Local communities of Oromo, Somali, and Afar make use of different species of *Aloe* for various purposes like, traditional medicine of both human and livestock, food, and cosmetics (Sebsebe *et al.*, 2000; Anteneh *et al.*, 2012).

The leaf latex of *A. trichosantha* (the sub species was not specified) has been used for treating stomach ailments, gastro-intestinal problems, skin diseases, wounds, and burns ([Anwar et al., 2014](#)). *Aloe harlana* was reported to be used for treating swollen body part locally called *gofla* ([Anteneh and Negussie, 2014](#)). *Aloe pirottae* is valued as a remedy for malaria, tropical ulcer, gastro-intestinal parasites, gallstone, eye diseases and snake bite. The leaf extract is dried and ground material called *sibri* (Oromo language) sold in the local markets of eastern Ethiopia, which was acclaimed as a cleaner of the human colon ([Anteneh et al., 2012](#)). The leaves of *A. trigonantha* is used for the treatment of infectious and inflammatory diseases ([Mekdes et al., 2015](#)). The limited ethnobotanical reports on *Aloe* species were focused on traditional medicinal values from leaf exudates. These medicinal properties of plants are normally dependent on the presence of certain phytochemical principles such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols, which are the bioactive bases responsible for the therapeutic properties ([Mann et al., 2008](#)).

2.7. Phytochemical Constituents of Ethiopian Aloe

The phytochemical studies of some Ethiopian aloes revealed a number of phenolic compounds isolated from the different Aloe parts, mainly leaf exudates ([Anwar et al., 2014](#); [Mekdes et al., 2015](#)). For example, phytochemical investigation on *A. calidophila* resulted in the isolation of three anthrones identified as aloinoside, aloin, and microdontin on the basis of IR, MS, ¹H NMR, and ¹³C NMR spectral data ([Abeje et al., 2014](#)). Two new compounds, dihydroisocoumaringlucoside (coumarin) and 6'-malonylnataloin (anthrone) were confirmed in this work. The isolated compounds showed strong antileishmanial (against *L. aethiopica* and *L. major*) activity and aloinoside was reported as better to arrest *L. aethiopica* and *L. major* activity than the standard drugs for Leishmaniasis ([Abeje et al., 2014](#)). Some phytochemical studies from the extracts of leaves, roots and rhizomes of *Aloe* species resulted in chemical compounds of chemotaxonomic value like, plicataloside from *A. otallensis* ([Viljoen et al, 1999](#); [Dagne et al., 2000](#); [Viljoen et al., 2001](#)).

Even though most *Aloe* species share one to three compounds between and among themselves, indicating some degree of relationships in their chemical composition, much variation were also

observed in the distribution of typical aloe compounds in leaf exudates. Aloenin, aarbaloin, nataloin, aloinoside, homonataloin, 7-Hydroxyaloin, aloesin and microdontin were reported among and between different species like, *A. debrana*, *A. calidophila*, *A. camperi*, *A. elegans*, *A. sinana*, *A. megalacantha*, *A. pubescens*, *A. pulcherrima*, *A. riviae*, and *A. secundiflora* (Dagne and Alemu, 1991; Dagne, 1996). For example, *A. pulcherrima* uniquely showed Nataloin and 7-Hydroxyaloin as its major constituents (Dagne and Alemu, 1991). The root-chemistry of some investigated species like, *A. calidophila*, *A. camperi*, *A. gilbertii*, *A. riviae*, *A. pulcherrima*, *A. schelpei*, *A. sinana* and *A. megalacantha* was found more similar in possessing aloechrysone, aloesaponarin I, aloesaponol I, aloesaponarin II, aloesaponol II, aloesaponol III, asphodelin, chrysophanol, chrysophanol-8-methyl ether, helminthoparin, and laccaic acid D-methyl ester, except *A. secundiflora* differ in that it does not contain aloechrysone (Dagne and Alemu, 1991; Dagne et al., 1994). In the root extract of these species, the chemotaxonomically important 2-naphtoic acid derivative, isoeleutherol, might serve as chemotaxonomic marker for the group (Dagne et al., 1994). Such studies have shown that chemical data are useful in providing evidences to taxonomic revision of the genus *Aloe* in the flora area.

Recent studies focused on the medicinal importance of the diverse phytochemical compounds from the leaf exudates of many Ethiopian *Aloe* species. *A. harlana*, *A. pulcherrima*, *A. sinana*, *A. trigonantha*, *Aloe trichosantha* were reported for possessing bioactive compounds of antimicrobial activities (Gelila et al., 2011; Anwar et al., 2014; Genet et al., 2014, Mekdes et al., 2015); *A. citrina*, *A. otallensis*, *A. gilbertii*, *A. pirottae*, *A. macrocarpa*, *A. pulcherrima* for antiprotozoal activities (Damtew et al., 2014, Yitagesu et al., 2014; Biruktawit et al., 2015; Dele et al., 2017); and *A. yavellana* was reported for larvicidal activities (Tibebu et al., 2018). Though comprehensive works are still lacking on the phytochemistry of Ethiopian Aloes, the limited works done so far on some *Aloe* species indicated that Ethiopian species possess diverse chemical constituents as well as unique types of compounds, especially the leaf exudates (Table 5), which could have potential for pharmaceutical application compatible with the current commercialized *Aloe* species.

Table 5. The diversity of phytochemical compounds identified from Ethiopian Aloe species using different chromatographic and spectroscopic techniques.

No .	Aloe species/endemicity	Part used	Method used	Phytochemical constituents	Source
1	<i>Aloe calidophila</i>	Exudate	HPLC; preparative TLC, 1H, 13C-NMR and MS	aloinoside, aloin, and microdantin (anthrones); Dihydroisocoumaringlucoside, aloin A/B, aloinoside A/B, microdantin A/B	Viljoen et al., 2001 ; Abeje et al., 2014
2	<i>Aloe camperi</i>	Exudate	HPLC	aloinoside, aloin, and microdantin (anthrones); Dihydroisocoumaringlucoside, aloin A/B, aloinoside A/B, microdantin A/B, unidentified anthrone	Viljoen et al., 2001
3	<i>Aloe citrina</i>	Exudate	Preparative TLC, 1H, 13C-NMR and MS	Homonataloin A/B	Biruktawit et al., 2015
4	<i>Aloe debrana</i>	Leaf & root	NMR, IR spectra, UC spectra	Chrysophanol, B-sitosterol, aloin-emodin, barbaloin, aloinoside, aloechryson	Dagne and Alemu, 1991
5	<i>Aloe elegans</i>	Exudate	HPLC	aloinoside, aloin, and microdantin (anthrones); Dihydroisocoumaringlucoside, aloin A/B, aloinoside A/B, microdantin A/B, unidentified anthrone	Viljoen et al., 2001
6	<i>Aloe ellenbeckii</i>	Leaf	HPLC-PDA and mass spectrometric analysis (LC-UV-MS)	6'-Malonylnataloin (rare anthrone nataloin)	Grace et al., 2008
7	* <i>Aloe gilbertii</i>	Exudate	HPLC; TLC, NMR spectra; acid hydrolysis of aloin A/B	Aloin, Aloe-emodin(Orange crystals) and Rhein (pale yellow powder); Aloesin, 7-O-methylaloesin, aloin A/B, aloinoside A/B, microdantin A/B	Viljoen et al., 2001 ; Yitagesu et al., 2014
8	<i>Aloe harlana</i>	Exudate	HPLC; Preparative TLC and NMR spectra	Aloin A/B, aloinoside A/B, microdantin A/B; anthrone (aloin) and chromone (7-O-methylaloesin A)	Viljoen et al., 2001 ; Gelila et al., 2011
9	<i>Aloe jacksonii</i>	Leaf	TLC and HPLC	Homonataloin A/B	Dagne et al., 2000
10	<i>Aloe macrocarpa</i>	Exudate	Preparative TLC and oxidative hydrolysis of aloin A/B	Aloin A/B, microdantin A/B, aloinoside A/B; aloin-emodin and rhein are semisynthesized derivatives of aloin A/B; Aloinoside,	Yitagesu Tewabe et al., 2019
11	<i>Aloe mcloughlinii</i>	Exudate	HPLC	Aloesin, dihydroisocoumaringlucoside, aloeresin D, aloin A/B, aloinoside A/B	Viljoen et al., 2001
12	* <i>Aloe megalacantha</i>	Leaf	HPLC	Dihydroisocoumaringlucoside, aloin A, aloin B, aloinoside A, aloinoside B, microdantin A, and microdantin B, aloin-emodin, barbaloin	(Dagne & Alemu, 1991 ; Viljoen et al., 2001)
13	<i>Aloe otallensis</i>	Exudate	HPLC; Preparative TLC and NMR spectra	2,8-O,O-di (β -D-glucopyranosyl)-1,2,8-trihydroxy-3-methylnaphtalene(plicataloside); Plicataloside	Viljoen et al, 1999 ; Biniam et al., 2011
14	<i>Aloe percrassa</i>	Leaf exudate	preparative TLC over silica gel, UV, MS, 1H, and 13C NMR spectrum	aloin A/B and microdantin A/B	Gerezihir Geremedhin et al., 2014

15	<i>Aloe pubescens</i>	Gel	HPLC	acetylated mannans, polymannan, aloin and alinoside, Aloesin and Aloeresin	Viljoen et al, 1999
16	<i>Aloe pulcherrima</i>	Root	Analytical TLC and NMR spectroscopic techniques	Chrysophanol, aloesaponarin I, aloesaponarin II, nataloin, 7-hydroxybarbaloin, B-sitosterol	Dagne & Alemu, 1991; Abdissa et al., 2017
17	<i>Aloe rivaer</i>	Leaf	TLC and HPLC	Dihydroisocoumaringlucoside, aloin A, aloin B, aloinoside A, aloinoside B, microdantin A, microdantin B, aloe-emodin, barbaloin	Dagne & Alemu, 1991; Viljoen et al., 2001
18	<i>Aloe rugosifolia</i>	Exudate	HPLC	Plicataloside	Viljoen et al, 1999
19	<i>Aloe ruspoliana</i>	Exudate	TLC and HPLC	Alkaloids, 2"-O-p-coumaroyl aloenin, nataloe emodin-7-glucoside, unidentified compound	Oketch-Rabah, 1997
20	<i>Aloe secundiflora</i>	Leaf, exudate	HPLC	Aloenin, aloenin B, isobarbaloin (aloin B), barbaloin (aloin A), aloinside A, aloinside B, aloesin derivative, unidentified mixture of dimers ; Aloenin, aloin B, and aloin A	Viljoen and VanWyk, 2000; Rebecca et al., 2003
21	<i>Aloe sinana</i>	Exudate	preparative TLC, 1H, 13C-NMR and MS	Aloin, aloinoside, microdantin (anthrones)	Genet Minale et al., 2014
22	<i>Aloe steudneri</i>	Exudate	HPLC	Dihydroisocoumaringlucoside, 8-Omethy-7-hydroxyaloin, aloin A, aloin B, microdantin A, and microdantin B	Viljoen et al., 2001
23	<i>Aloe tewoldei</i>	Exudate	HPLC	Dihydroisocoumaringlucoside, aloin A, aloin B, aloinoside A, aloinoside B, microdantin A, and microdantin B	Viljoen et al., 2001
24	* <i>Aloe trichosantha</i>	Exudate	Preparative TLC, ESI-MS, and NMR	Aloin A/B (1); aloin-6'-O-acetate A/B (2) = new	Anwar et al., 2014
25	<i>Aloe trigonantha</i>	Exudate	Analytical RP-HPLC and preparative TLC	Aloesin (chromone) and 8-Omethy-7-hydroxyaloin A/B, aloin A/B, aloin-6'-O-acetate A/B (anthrones)	Mekdes et al., 2015
26	<i>Aloe yavellana</i>	Exudate	Preparative TLC, NMR and ESI-MS	aloin A/B and microdantin A/B	Tibebu et al., 2018

HPLC = High performance liquid chromatography, TLC - Thin layer chromatography; ESI = Electro-spray ionization; MS = mass spectrometry; RP = Reverse phase; NMR = IR spectra, UC

*Note: The phytochemical studies conducted on *A. gilbertii*, *A. megalacantha*, and *A. trichosantha* did not specified the subspecies in which these three species have two subspecies each.

3. MATERIALS AND METHODS

3.1. Study Area

The data in this PhD dissertation research was collected from the eastern, south-eastern, and southern floristic regions of Ethiopia. The focus was in the east and south of the Great Rift Valley floristic regions that included Afar (AF), Arsi (AR), Bale (BA), Hararge (HA), and Sidamo (SD) floristic regions considering the rift valley as reference line (Figure 1/map in article I). The specific study sites in the respective floristic regions are all listed (Appendix table 3).

3.2. Plant Collection and Authentication

Fresh leaves, inflorescence, and seedlings of targeted *Aloe* species were collected from five floristic regions in their corresponding natural habitats. Their taxonomic identification and authentication were done in the National Herbarium of College of Natural Sciences, Addis Ababa University (AAU) using taxonomic keys in the Flora of Ethiopia and Eritrea (Edwards *et al.*, 1997) as well as through visual comparisons with authenticated plant specimens kept at the National Herbarium (ETH). Taxonomic identities were confirmed by Sebsebe Demissew (Professor of Plant Systematics and Biodiversity). Voucher specimens were deposited at AAU and Haramaya University (HU) Herbaria as well as their seedlings were planted at the garden in HU.

The fresh leaves of each *Aloe* species were used for exudate extraction by cutting the leaves transversally near the base and arranging them concentrically around plastic plates to drain exudate for 10-20 minutes. Then exudates of each species were poured in airtight plastic bottles, kept in field ice bag and brought to Lab for storage in the refrigerator at 4° C until proceed to freeze-drying. The extracts were protected from any possible oxidation by being stored in airtight aluminium foil covered flasks. Then each exudate were freeze dried (Christ Alpha 1-2 LDplus freeze drier, Germany) to be crystallized and powdered, labelled and stored at 4° C until used for phytochemical analysis and toxicity test.

3.3. Experimental Animals

Outbred female, nulliparous, and non-pregnant Swiss albino mice aged 6–8 weeks and weighing 23–30 g were used for toxicity test experiment. Newly born female mice at the age of 21 days were separated in sexes and housed in polypropylene cages with stainless steel wire mesh cover ($n = 5/\text{cage}$). All groups were kept under 12 hours light and dark cycles at ambient room temperature (22–25° C) of the toxicology laboratory of Haramaya University with free access to standard rodent pellet diet and water *ad libitum*. Mice were acclimatized to the laboratory conditions for one week before initiation of the experiment. All procedures complied with the care and use of laboratory mice was in accordance with the guideline for the care and use of experimental animals stated in OECD 425 (OECD, 2008). Experimentation ethics committee on animal use of the school of animals and range sciences of Haramaya University was approved the protocol used in this study.

3.4. Chemicals

The major chemicals used were acetonitrile (CH_3CN), chloroform/trichloromethane (CHCl_3), ethyl acetate/EtOAc ($\text{C}_4\text{H}_8\text{O}_2$), methanol/MeOH (CH_3OH), formic acid (CH_2O_2), potassium hydroxide (4% KOH), 2-aminoethyl diphenyl borinate (1.5gm) in 150 ml MeOH, and polyethylene glycol 6000 (7.5 gm) in 150 ml ethanol for natural product derivatizer preparation and ultrapure water purified with a NaNo pure Diamond UV system (Barnstead International, Dubuque, IA, USA). All chemicals used for mobile phase were HPTLC and UHPLC analytical grade from Burdick & Jackson (Muskegon, MI, USA) and from Sigma-Aldrich (St. Louis, MO, USA).

Standards used in optimization of UHPLC-MS/MS and HPTLC parameters were also standard grades from Tshwane University of Technology (TUT), Department of Pharmaceutical Sciences laboratories where this experiment were performed in Pretoria, South Africa. Available standards were used like, aloin A and B, aloinoside A and B, microdontin A and B, and homonataloin A and B. Others like, aloe-emodin, dihydroisocoumarin, aloeresin A, aloeresin D, and aloeresin G were used from TUT, which have been isolated from *Aloe ferox*, *A. dolomitica*, *A. marlothii*, and *A. thraskii* (Provided by Prof. Alvaro Viljoen).

3.5. Equipment

3.5.1. HPTLC Equipment

The HPTLC system was composed of CAMAG supplies such as, automatic TLC sampler 4 (CamagMuttenez, Switzerland) with the syringe, 100 μ L (Hamilton Bonaduz, Switzerland) spotting device (ATS 4) ; the densitometer consisted of CAMAG TLC scanner-3 provided with visionCATS HPTLC software (version: 1.4.4.6337), automatic developing glass twin trough chamber (20 x10 cm); automated derivatizer, TLC plate heater II, and TLC visualizer 2, and pre-coated silica gel 60 F₂₅₄ HPTLC plates (20 cm x 10 cm; with 0.25 mm thickness) with aluminium sheet support (Figure 5).

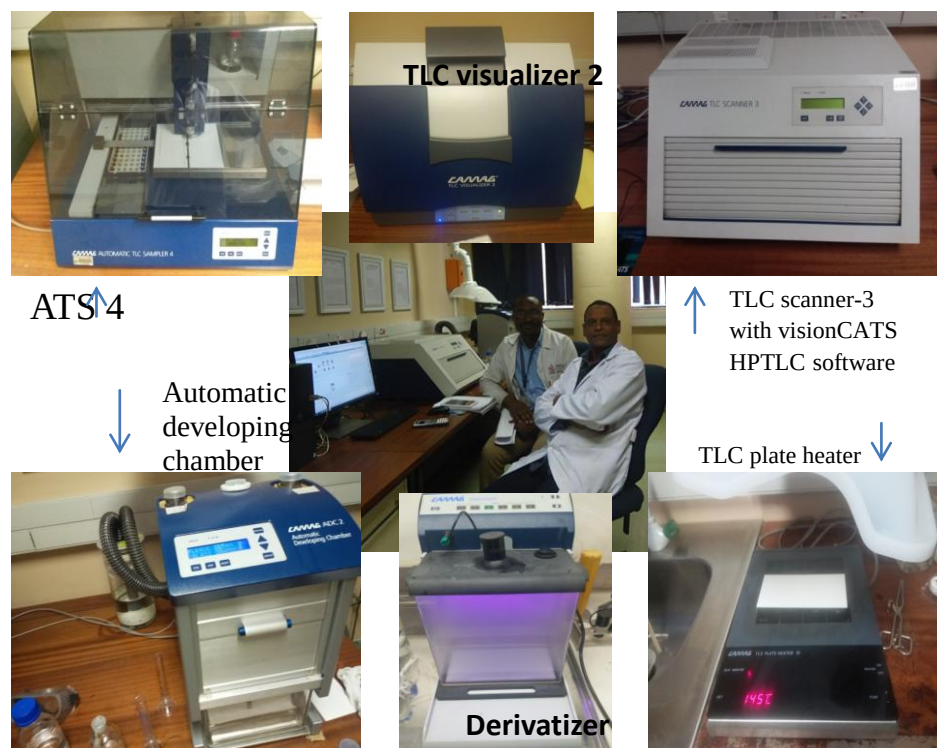


Figure 5. The CAMAG HPTLC instruments used for automated TLC fingerprinting and chromatogram development (Department of pharmaceutical sciences laboratory of Tshwane University of Technology, Pretoria, South Africa).

3.5.2. UHPLC-MS-DAD Equipment

An ACQUITY ultra-high performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS) and ultraviolet-photodiode array detection (UV-DAD) system with Masslynx (version 4.2) software (Waters®) was used. This system consisting of the following components: ACQUITY UHPLC BEH C18 Column (150mm x 2.1 column-Ireland; particle packing size 1.7 μm); Agilent Technologies 7693 Autosampler; Waters Zspray; ACQUITY sample manager; ACQUITY PDA $\text{e}\lambda$ detector (Waters Acquity PDA detector type was UPLC LG 500 nm, bandwidth 1.2 nm); and Isocratic Solvent Manager and QDa Detector ($\text{ESI}^\pm$, cone: 15 V, capillary: 0.8kV, 150 to 850 m/z) (Figure 6).

3.5.3. Equipment for Acute Oral Toxicity Test

Freeze drier (Christ Alpha 1-2 LDplus), polypropylene cages with stainless steel wire mesh, rodents dissecting kit with complete set, dissecting board, and oral gavage (18 gauge).



Figure 6. The complete set of UHPLC-MS-DAD equipment (Department of pharmaceutical sciences laboratory of Tshwane University of Technology, Pretoria, South Africa).

3.6. Sample Preparation

3.6.1. Sample for HPTLC

A 30mg exudate sample in 3mL methanol (HPTLC grade MeOH) was dissolved and sonicated (40 kHz, 105 W) at room temperature using an ultra-sound machine (Mujigae, Seoul, Korea).

The resultant dissolved solution was transferred to 2 ml vial for CAMAG HPTLC auto-sampler. The rest 1 ml sample was used for repeated manual TLC for mobile phase optimization.

3.6.2. Sample for UHPLC-MS-DAD

A portion of each *Aloe* exudate (250 μ g) was dissolved in 1mL of 0.1% formic acid in 100% acetonitrile filtered through a 0.45 μ M micropore membrane (PTFE, Waters Milford, MA, USA) before use. Later, it was injected into the UHPLC-PDA and ESI-Orbitrap-MS equipment with an injection volume of 1.00 μ L.

3.6.3. Sample for Acute Oral Toxicity Test

Powdered exudate of each *Aloe* species was dissolved in sterile distilled water to prepare two doses of 300 mg/kg and 2000 mg/kg body weight. Doses were calculated in reference to the body weight as the volume of the exudates powder solution given to the mouse was 1mL/100 g.

3.7. Ethnobotanical Data Collection

Comprehensive data were collected on the ethnomedicinal and bio-cultural values, including population trends of species in the natural habitat from the emic perspective, and associated indigenous knowledge in the different seasons from 2017 (March to April and October to November) to 2018 (January to February and June to July). A total of 210 respondents (152 men and 58 women) from four cultural communities (Oromo, Somali, Afar, and Harari) participated. Informants were selected by stratified random sampling techniques (randomly selected informants/RI) and purposive sampling techniques to systematically select the traditional healers (TH/key informants) who have in-depth traditional knowledge concerning multi-utility of *Aloe* species of their localities. Age, gender, and occupation (Article I) were considered, as suggested by [Martin \(1995\)](#) and [Caruso et al, \(2015\)](#).

Semi-structured interviews, focus group discussion (FGD), practical observation sessions, and guided field walk in *Aloe* localities were conducted after Oral Prior Informed Consent (PIC) was sought from every respondent. Most interviews were conducted in the field in order to avoid the risk of confusing identity of *Aloe* species. The respondents were asked to freely list all possible

uses of each *Aloe* species, each time a plant was mentioned as “used” was considered as one “use-report”, and repeated mention of same use-report by different informants was taken as “use mention or number of citation” (Martin, 1995; Cunningham, 2001). A total of ten FGD were conducted having 7-11 members of men and women per group. Data recorded were: vernacular names of *Aloe* species, local uses, parts used, ingredients added during the use formulations (if any), and locally produced and marketable aloe products. For medicinal use-reports, the ailments treated, preparation procedures, method of administration, and antidote (if any) were recorded. In addition, population trends (noticeably increasing, increasing, noticeably decreasing, decreasing, stable, and not sure/uncertain) from emic perspective were recorded (Appendix table 3). Finally, the use-reports recorded were categorized into major use categories (UC) of level 1 and sub-categories of level 2 using the Economic Botany Data Standard (Cook, 1995).

3.8. HPTLC Experimental Procedure

The phytochemical analysis procedure was started with cleaning HPTLC plates by predevelopment with methanol in ascending method. Then the vials containing samples and HPTLC plate of 20 cm × 10 cm HPTLC silica gel 60 F₂₅₄ were immersed in a CAMAG Automatic TLC Sampler IV glass chamber (20 cm × 10 cm), containing 30 mL methanol (HPTLC grade) as solvent system for automated spotting. Samples were administered to the HPTLC plates as 8 mm band width, 3 mm between bands, and 8 mm from the bottom of the plate via Linomat-5 automatic sample applicator equipped with a 100 µl Hamilton syringe. Volumes applied was 2.0 µL with the syringe rinsed with absolute methanol between samples at delivery rate of 100 nl/s (Loescher *et al.*, 2014; Pandeya *et al.*, 2016).

After complete spotting, the plate was removed from TLC glass chamber and dried in an oven at 105⁰ C for 5 minutes then was put in developer, which consistent of the mobile phase; ethyl acetate (EtOAc): Acetonitrile (CH₃CN): formic acid (CH₂O₂) (9:2:1 v/v/v) selected after several optimization trial. Once development was completed (Chromatograms were developed for 9.0 cm) in Camag Automatic Developing Chamber (ADC-2), a photo was taken with a 'TLC visualiser 2' under white light and UV wavelengths of 254 nm and 366 nm before derivatization. Then plate was derivatized by spraying 4% potassium hydroxide in methanol (96% MeOH)

using Camag Derivatizer and heated a plate on Camag TLC plate heater at 100 °C for 5 minutes. Finally, densitometric screening was performed by using Camag TLC Scanner IV at 366 nm and VisionCATS software in fluorescence mode after derivatization. The results were observed and captured under white light and 366 nm under Camag TLC visualizer 2 (Loescher *et al.*, 2014; Ojha and Kumar, 2012; Pandey *et al.*, 2016).

3.9. UHPLC/ESI-MS-MS -DAD Experimental Procedure

Ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis coupled with ultraviolet-photodiode array detection (UV-DAD) were performed on a Waters® Acquity UHPLC system (Waters, Milford, MA, USA) equipped with electrospray ionization (ESI), photodiode array (PDA) detector/UV spectrum in the negative mode, an auto sampler, and column oven for identification and characterization of secondary metabolites in the targeted *Aloe* species. The chromatographic separation was carried out using a Waters® Acquity UHPLC BEH C18 column; 2.1 mm x 150 mm; 1.7µm particle packing size (Waters-Ireland), which provide higher resolution and better efficiency than the conventional columns (Novakova *et al.*, 2006; Meier *et al.*, 2017).

The column temperature was set to 40 °C during operation and target sample temperature was 25 °C. A five-minute gradient program was optimized at a flow rate of 0.5 mL min⁻¹ with an injection volume of 5 µL. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow rate of 0.30 ml/min. The gradient parameters/elution were as follows: 00 min, 0.3 flow rate from 95% A and 5% B, 13.5 min, 0.3 flow rate from 60% A to 40% B, 14 min, flow rate 0.3, from 20% A to 80% B, 14.5 min, flow rate 0.3, from 95% A to 5% B (back to initial ratio). The injection volume was 1.0 µL (full-loop injection). Data were collected between 100 and 1500 m/z. The LC eluent was introduced directly into the electrospray ionization source without splitting the flow of the eluent. The chromatograms were recorded at wavelength of 287 nm while the UV spectra were monitored over a range of 400–200 nm. (Chen *et al.*, 2009; Cao *et al.*, 2009; Epriliati *et al.*, 2010; Lamuela-Raventós *et al.*, 2014).

3.10. Acute Oral Toxicity Test

Acute toxicity describes the adverse effects of a test substance resulted after administration of either from a single dose or multiple doses to a given concentration within 14 days of the administration of the substance (McNaught and Wilkinson, 1997). In this study, acute oral toxicity test of the leaf exudate powder from 17 *Aloe* species were evaluated on a total numbers of 35 groups (17 *Aloe* species x 2 doses/concentrations and one negative control group of distilled water), with a total of 210 mice. Initial body weight of each mouse was recorded after the fasting period of 3 hrs.

Powdered exudate of each *Aloe* species was dissolved in sterile distilled water and administered in a single loading dose of 300 and 2000 mg/kg body weight at a rate of 1mL/100 g orally by oral gavage (18 gauge). Whereas, the control group received sterile distilled water at the same rate of test substance. Food and water were provided an hour after dosing. Following oral administration of *Aloe* exudates, mice were observed individually and periodically during the first 24 hours (0.5, 1, 2, 4, and 12 hrs) with special attention given to the first 4-6 hours, and daily thereafter for a total of 14 days (OECD, 2001). Then toxic symptoms and behavioural changes such as, piloerection, sedation or sleep, hyperactivity, restlessness, writhing, tremor, convulsion, lethargy, salivation, lacrimation, loss of appetite, diarrhea, and mortality were monitored and recorded accordingly (Camargo *et al.*, 2004; Boudreau and Beland, 2006). The median lethal dose (LD₅₀ oral [p.o.]) was estimated based on mortality recorded within 24 hrs and the body weights of the mice were measured on days 7 (week 1) and 14 (week 2) following OECD guideline 423 (OECD, 2001). Then two week after, all animals were euthanized by CO₂ inhalation at the end of the experiment, and their major organs (liver, kidney, heart, and spleen) were removed, cleaned, observed for gross pathology and measured.

3.11. Data Analysis

3.11.1. Ethnobotanical Data Analysis

Ethnobotanical data were organized and cleaned in an excel spreadsheet (Microsoft office 2010), to be suitable for both qualitative and quantitative analysis. Qualitative data were analyzed following Martin (1995), Cotton (1996), and Cunningham (2001) such as, free listing, part used, and use-reports. Quantitative data were analyzed using the corresponding formulas as follows for

Frequency of citation for use-report (FC_{UR}), Relative Frequency of Citation (RFC_s) (Tardio and Pardo-De-Santayana, 2008), Informant consensus factor (icF) (Trotter and Logan, 1986; Heinrich, 2000), Use value (UV), Relative Importance Index (RI) (Tardio and Pardo-De-Santayana, 2008) and Cultural Value Index (CV) (Reyes-García *et al.*, 2006) (detail in Article I).

Nonparametric Kruskal Wallis Test was performed for correlation analysis between age of informant vs. knowledge on use of aloes (number of use-reports). Non-parametric pair wise Wilcoxon test was performed to test significant difference between informant's gender and knowledge about use of aloes, using R software version 3.3.4 using multcompview and R companion packages. P-values of less than 0.05 were taken as statistically significant difference.

3.11.2. Phytochemical Data Analysis

HPTLC data were analyzed using VisionCATS software fixed with Camag TLC Scanner IV. The chemometric data of the UHPLC-MS- diode array/UV spectrum data were analyzed using MassLynx (version 4.2) software. Peaks were identified by comparing retention times, pseudo molecular ions mass, and UV spectra as well as compared with some reference standards. A binomial test was run using the R package OneTwoSamples (Zhang, 2013) to evaluate the mean difference in number of visible bands before and after derivatization. P-value less than 0.05 was taken statistically significant difference.

3.11.3. Toxicity Data Analysis

Toxicity test data were presented as mean $\pm$ SEM with 95% confidence interval for mean percentage body weight change, final body weight gain, mean relative organs' weight change in relation to absolute final body weight and food and water consumption. The relative organ weight was calculated as absolute organ weight (g)/body weight of rat on the sacrifice day (g) $\times$ 100. The percentage of body weight change was calculated as body weight at the end of each week minus initial body weight divided by initial body weight multiplied by 100. Then one-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons of the mean differences of the two doses of *Aloe* exudates compared to the negative control. P value of less than 0.05 was taken as statistically significant difference compared to the negative control.

4. SUMMARY OF RESULTS

4.1. Ethnomedicinal and Bio-cultural Values of Aloes

A total of 23 *Aloe* species were comprehensively studied for ethno-medicinal and bio-cultural values (Article I). Among these 16 *Aloe* species were phytochemically analyzed (Paper II and III) and their toxicity effect were studied (Article IV). The total number of 196 use-reports, which were categorized into six major use categories ($N_{UC}=6$) with 2158 citations (use mentions), were documented from the 23 *Aloe* species by 210 respondents. The major use categories (UC) were medicines (Md), social uses (SU), materials (Mt), environmental uses (EU), vertebrate poisons (VP), and food (Fd). Among these major use categories, medicinal use category accounted 149 use-reports (76%) from 22 *Aloe* species with 1607 citations. The highest number of use-reports was recorded for *Aloe megalacantha* subsp. *alticola* with 23 use-reports followed by *A. trichosantha* subsp. *longiflora* with 20 use-reports and *A. calidophila* with 18 use-reports.

A total of nine different plant parts were used for the diverse ethnomedicinal and bio-cultural uses. Leaf exudate was the most frequently sought *Aloe* product, accounting for 111 use-reports (49.1%) in which about 89 (80.2%) were for human and 12 (10.9%) were for livestock medicinal formulations. That means, 91.1% of exudate were used for medicinal purposes and the remainder formulations were applied in social uses and vertebrate poisons categories, which is a unique use-report for *Aloe retrospiciens* and *A. ruspoliana*. The highest frequency of citation was 100% recorded for leaf exudate of *Aloe otallensis* used for weaning children from breast-feeding followed by 94.4% for leaf exudate of *A. yavellana* used to treat jaundice, 91.6% for leaf gel of *A. rivae* used for body and hair wash, and 90.9% for exudate of *A. megalacantha* subsp. *alticola* to treat skin infections.

The local communities practiced nine types of preparation/formulation methods for medicinal applications. Out of these, 66 (42%) were prepared in the form of pure exudate collection from fresh leaf to be used for different applications followed by pulverization (11.5%) and concoction (10.8%).

4.2. Ethnomedicinal and Bio-cultural Knowledge

The highest number of use-report in all major use categories were reported by elderly people (above 60 years, N = 35). For example, out of the total 1336 use mentions for human medicinal uses, 654 were reported by elderly people, followed by 398 use mention reports by adults (age 41-60, N = 108) and 284 by young informants (age 25-40, N=67). The depth of comprehensive bio-cultural knowledge among different age groups indicated that elderly people had deeper knowledge than the two age group ranging from 25 to 40 and 41-60 (Kruskal-Wallis chi-squared = 12.17, df = 3, $p=0.006^*$). The significance of difference in knowledge depth is higher between age categories of young and elderly people ($p=0.0004^{**}$) than between adult and elderly people ($p=0.02^*$) ($p<0.05$). In addition, men were more knowledgeable on identifying *Aloe* at the lower taxonomic level and diverse medicinal use-reports than women (Wilcoxon test, $p=0.002$), which is significantly different ($p<0.05$). Women were found more knowledgeable than men for cultural uses like, cosmetic (Wilcoxon test, $p=0.06$), not significantly different ($p>0.05$).

Informant consensus factors (F_{ic}) of the six major use categories ranged from 0.86 to 0.22. The highest F_{ic} value was 0.86 for medicines in 11 use sub-categories from 22 species, followed by 0.63 for environmental uses in three sub-categories from four species and the least F_{ic} value was 0.22 for materials in two sub-categories from eight species. *Aloe* species were compared based on the values of importance metrics. Species like *Aloe calidophila*, *A. megalacantha* subsp. *alticola*, *A. pirottae*, and *A. gilbertii* subsp. *gilbertii* showed higher values across the indices due to being mentioned by higher number of informants, relatively higher number of use-categories, and larger number of use-reports to elucidate species that were of high importance in all indices led to further phytochemical and toxicity analysis.

4.3. HPTLC Detection and Isolation of Bioactive Compounds

The mobile phases are determinant factor for accurate spotting of separated bands (analytes compounds) in all chromatographic techniques. That is why in this study different mobile phases and derivitizers were tested for better separation of the bands. The mobile phase ethyl acetate: acetonitrile: formic acid in the volume ratio of 9:2:1 v/v/v with 4% KOH in 96% MeOH derivatizer resulted in well-resolved and much better bands with distinct R_f values (Paper II). To

the knowledge of the authors, this mobile phase has not been used so far for the phytochemical analysis of Ethiopian *Aloe* species with HPTLC system.

The HPTLC chromatogram produced a number of well-resolved bands with different retention factor (Rf), which are less visible before derivatization under white light, UV 254 nm, and UV 366 nm and the number of visible bands were significantly increased after derivatization ($P < .05$) (Paper II). The maximum number of detected bands were 14 on track 3 (*A. pirottae*) and minimum were two on track 12 (*A. rugosifolia*). An innovative method of double development followed with derivatization and scanned under UV 366 increased the visibility, optimized the band separation, and revealed well-resolved bands along all tracks.

The isolation of bioactive compounds indicated that, like most *Aloe* species, the HPTLC bands were dominated with anthrone and chromones. In addition, few anthraquinones, coumarins, pyrans and pyrones, alcohol, and benzene, naphthaline and furan derivatives were isolated. The most common anthrones like, aloin A and B, aloinoside A and B, and minicrodantin A and B as well as aloeresins (chromones) were isolated. There was no any band detected from the leaf exudates of *Aloe retrospiciens* and *A. ruspoliana*, which were reported as "poisonous to carnivores" by the informants.

4.4. UHPLC-MS Chromatograms for Phytochemical Characterization

The phytochemical characterization from leaf exudates of 14 *Aloe* species resulted in about 56 compounds of secondary metabolites, in which 35 were identified (62.5%) and 21 were unidentified (37.5%) using UHPLC-MS and DAD UV spectrum methods (Paper III). The characterized and identified compounds belonging into nine compound classes i.e. anthraquinones, anthrones, chromones, coumarins, benzene, naphthalene and furan derivatives, flavonoid, oxilipin, alcohol, and pyrans and pyrones. Anthrones and chromones were the dominant compound classes accounted for 65.7%, 12 (34.3%) anthrones and 11 chromones (31.4%), of identified compounds in the leaf exudates of targeted 14 *Aloe* species. Some unique and new compounds, which have not been reported so far from Ethiopian *Aloe* species such as 7-O-methylaloesin, trihydroxy octadecenoic acid, 7-Hydroxy-8-O-methylaloin B,

deacetylaloin, 6'-O-coumaroyl aloesin, 7-O-methyl-2'-feruloylaloesin, 1-hexanol-pentosyl hexoside, 1-O-methylpicalalloside, caffeoyl ester of aloesin, isorabichromone, and aloenin B were isolated from some of these targeted *Aloe* species (Paper III).

Aloe pirottae ranked first with 20 isolated compounds (15 were identified and 5 were unidentified) followed by *A. secundiflora* and *A. tewoldei* with 15 compounds each and *A. harlana* and *A. mcloughlinii* each with 14 compounds. *Aloe calidophila* and *A. rivaie* has each 13 compounds. The least number of compounds (four) were isolated from *A. rugosifolia*, in which 95 percent is constituted by a single compound plicataloside (a benzene, naphthalene and furan derivative). The laxative compound aloin A/B was found at higher concentration in the leaf exudates of *Aloe harlana* (47%), *A. megalacantha* subsp. *megalacantha* (40%), and *A. pirottae* (46%). The UHPLC-MS chromatogram peaks of two sub species, *A. megalacantha* subsp. *megalacantha* and *A. megalacantha* subsp. *alticola* showed much phytochemical variation found significantly different ($P < 0.05$), which could raise taxonomic discussion. Unlike other *Aloe* species targeted in this study, the leaf exudate samples of *Aloe retrospiciens* and *A. ruspoliana* collected and prepared in the same methods and conditions did not showed any compound.

4.5. Acute Oral Toxicity

4.5.1. General Signs and Behavioural Analysis of Oral Acute Toxicity Test

A single loading oral dose of leaf exudate from 11 *Aloe* species (*A. calidophila*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivaie*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora* and *A. yavellana*) did not induced any overt signs of toxicity and behavioural changes at the starting dose of 300 mg/kg body weight. However, mice administered with exudate from *A. calidophila*; *A. megalacantha* subsp. *alticola*; *A. megalacantha* subsp. *megalacantha*, *A. pubescens*, *A. rivaie*, *A. secundiflora*, *A. tewoldei*, and *A. yavellana* showed passive behavioural change at the next higher dose of 2000 mg/kg in the first 24 hrs and no mortality recorded from both doses. In addition, most groups, except groups treated with *A. otallensis*, *A. retrospiciens*, *A. rugosifolia*, *A. ruspoliana*, and *A. trichosantha* subsp. *longiflora* exhibited diarrhea 2-6 hrs post-administration of the exudates at 300 and 2000 mg/kg doses. Different degrees of diarrhea were noticed at same dose level among

different treatment groups in which exudates from *A. harlana*, *A. megalacantha* subsp. *megalacantha*, and *A. pirottae* induced high degree of diarrhea at both dose levels.

Groups treated with exudates from *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and unidentified/AHU53 induced slight behavioural changes at the minimum dose of 300 mg/kg body weight. Piloerection and restlessness were among the changes observed in few hours of post-administration. These symptoms were disappeared gradually after. Groups of mice (administered same four species above) treated with the maximum dose of 2000 mg/kg body weight showed passive behavioural and physiological changes such as piloerection, restlessness, tremors, and loss of appetite 2-4 hrs after oral administration up to 24 hrs of the assay with improvement day after day, which disappeared after 72 hrs. The leaf exudate of two near endemic *Aloe* species, *Aloe retrospiciens* and *A. ruspoliana*, showed overt signs of toxicity and behavioural changes at the doses of 300 mg/kg and 2000 mg/kg body weight. Deaths of 1-3 mice were also recorded in this treatment groups.

4.5.2. Absolute and Relative Body and Organs Weight Changes

Most *Aloe* species (except, *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and unidentified AHU53) showed no significant difference ($P>0.05$) of percentage change in mean absolute body weight at week 1 and week 2 compared to the control group (Article IV). Groups treated with exudate from *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and AHU53 exhibited significant difference ($P<0.05$) in percentage change of mean absolute body weight at week 1 and week 2 at both dose levels of 300 and 2000 mg/kg body weight compared to the control group.

Recordings of mean body weight gain on the fourteenth day showed significant different ($P>0.05$) from the control group at both dose levels for the test groups treated with exudate of *A. calidophila*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivaie*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora* and *A. yavellana*. However, it was significantly different ($P<0.05$) for groups treated with *Aloe harlana*, *Aloe mcloughlinii*, *A. pirottae* and unidentified/AHU53 at both dose levels, with negative values of between -3.14 and -0.04, compared to the control group.

Especially, the body weight gain was dropped more significantly for unidentified/AHU53, $-3.14 \pm .53$ ($P=.0001$), followed by *Aloe mcloughlinii* with $-3.1 \pm .53$ ($P=.0001$) at 2000 mg/kg dose, which are much significantly different ($***p<.0005$) from the control group (Article IV).

None of the treatment groups showed significance difference ($P>0.05$) in percentage weight of kidney, heart and spleen in relation to body weight or relative organs' weight (g%) at 300 and 2000 mg/kg body weight dose levels. However, percentage of liver weight in relation to the final body weight has showed significant difference ($P<0.05$) for groups treated with exudates of *Aloe harlana* and *A. pirottae* at 2000 mg/kg body weight whereas, groups treated with exudates of *Aloe mcloughlinii* and Unidentified/AHU53) were significantly different ($P<0.05$) at both dose levels. The food consumption had showed significant difference ($P<0.05$) for groups treated with exudates of *Aloe harlana* and *A. pirottae* at 2000 mg/kg body weight while groups treated with exudates of *Aloe mcloughlinii* and Unidentified/AHU53) were significantly different ($P<0.05$) at both dose levels of 300 and 2000 mg/kg body weight. The water intake of all treatment groups showed no significance different ($P>0.05$) at both dose levels compared to the control group.

5. GENERAL DISCUSSION

5.1. Ethnomedicinal, Bio-cultural Values, Phytochemistry and Toxicity Test

This study revealed that the common traditional uses of *Aloe* species were in the traditional health care system (76%). This compares closely with the value of 74% of literature-based use records describing the medicinal uses of *Aloe* (Grace *et al.*, 2009b) and 73% for human medicines from 11 *Aloe* species in Tanzania (Amir *et al.*, 2019). *Aloe* species were reported to have multiple uses with major uses for medicinal purposes (Akaberia *et al.*, 2016, Amir *et al.*, 2019). The more cited *Aloe* part used for the traditional medicinal purpose was *Aloe* exudate, predominantly used for skin and subcutaneous tissue ailments. The beneficial health-promoting properties present in the exudates of *Aloe* leaves were attributed to their diverse phytochemical composition (Cardarelli *et al.*, 2017). Phytochemical studies on the genus *Aloe* indicated that over 200 compounds belonging to different classes such as anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes, anthraquinones and flavonoids have so far been reported (Reynolds, 1985; Dagne, 1996; Ombito *et al.*, 2015). The HPTLC fingerprinting in this study revealed remarkable variation and diversity in the chromatographic profiles of targeted *Aloe* species, which could correspond with the reported diversified ethnomedicinal and bio-cultural applications through the synergetic actions of the compounds.

The higher frequency of citations of some use-reports can be explained by the fact that these *Aloe* species are renowned and have long been used by the majority of informants, representing a source of reliability. For example, the highest FC value (100%) for *Aloe otallensis* used for weaning child breast feeding could be attributed to extra effectiveness of its exudate. The 75% plicataloside concentration in *A. otallensis* could be attributed to its effectiveness for the purpose used. Additional new compounds like, 1-O-methylplicataloside and 7-O-Methyl-2'-feruloylaloetin, which have been isolated and characterized for the first time in this study from *A. otallensis*. This could have been maximized its value for the traditional purpose mentioned by the informants. Similarly, the local communities in Kenya claimed that *Aloe lateritia* is not as bitter as *A. secundiflora*, and hence not effective (Bjorå *et al.*, 2015). This indicates that the community has preference of specific *Aloe* species for the purpose, which could be attributed to

the phytochemical profile. The unique and new compounds isolated from the targeted *Aloe* species have revealed the potential therapeutic values for diverse ailments in the traditional health care system. Though comprehensive works are still lacking on the phytochemistry of Ethiopian Aloes, limited works done so far on some *Aloe* species indicated that they possess diverse chemical constituents as well as unique types of compounds (Dagne *et al.*, 2000).

It has been reported that some *Aloe* species have potential toxicity, with side effects occurring at some dose levels when taken orally or applied topically (Bottenberg *et al.*, 2007). Similarly, a unique use-report: "poisonous to carnivores" was reported from two near endemic *Aloe* species: *Aloe retrospiciens* and *A. ruspoliana*. These two *Aloe* species were also resulted in mortality of mice indicating that the species are highly toxic. Though there is no or single report on the phytochemical constituents of these two species so far, some alkaloids potentially toxic could be expected to pose the toxicity. Alkaloids, especially the ones belonging to the hemlock class, have been reported in some toxic species such as *A. sabaia* (Blitzke *et al.*, 2000; Reynolds, 2005). In Kenya and Somalia, it was reported that *A. ruspoliana* is used to kill hyenas by smearing meat with the leaf extract (Reynolds, 2004). Unfortunately, this study could not identified any constituents from *Aloe retrospiciens* and *A. ruspoliana*. Either the solvent systems used for HPTLC and UHPLC-MS-DAD techniques in this study did not work for these samples or the collected samples were failed for unidentified reasons. These two species could attract further studies, which may lead to taxonomic discussion. Further phytochemical analysis with different methods and design and molecular analysis are strongly recommended for these two *Aloe* species.

The majority of the young and adult informants were found to be less knowledgeable about the diversity of *Aloe* species and their uses compared to elderly informants. This could be attributed to the current cultural transformation and increasing accessibility of education and health centers in rural areas that has challenged the acquisition of indigenous knowledge among young people. An alternative interpretation is that knowledge accumulates over time, and that older people have had longer to learn about plant uses. Similar results were reported in some other cultural groups in Ethiopia (Yineger *et al.*, 2008; Anteneh and Bussa, 2014) and among users of *Aloe* species in Tanzania (Amir *et al.*, 2019). Some studies indicated that valuable ethnomedicinal information was shared with researchers mostly from informants over 60 years of age (Sargin and

Buyukcengiz, 2019). The deterioration of traditional knowledge of medicinal and bio-cultural values of most *Aloe* species along the younger generation could have negative consequences for their future conservation.

The most popular indices used to evaluate the relative importance of different species, which are used in the traditional system are based on “informant consensus,” i.e., the degree of agreement among the various interviewees (Albuquerque *et al.*, 2006; Moerman, 2007). In this respect, there was a strong consensus for medicinal values of the leaf exudate having higher Fic value. Efficacy of traditional medicinal plants is strongly correlated with Fic value, meaning pharmacologically effective remedies are expected to have greater Fic value, and vice versa (Trotter and Logan, 1986). The species with higher values in all indices of UV, RFC, RI, and CV like, *A. calidophila*, *A. megalacantha* subsp. *alticola*, and *A. pirottae* were identified to be the most ethnomedicinal and bio-culturally important species. The common anthrones and chromons like aloin A and B, aloinoside A and B, homonataloin A and B, microdontin A and B and aloeresin, which were characterized from these *Aloe* species must have contributed to their effectiveness. These compounds are the major constituents of commercial *Aloe* species like *A. ferox* and *A. vera* (Pandeya *et al.*, 2016; Sánchez-Machado *et al.*, 2017; Salehi *et al.*, 2018).

Additional compounds like, 3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one (feralolide-3'-O- β -D-glucopyranoside), aloemodin, kaempferol-3-O-hexosyl-O- pentoside, and *p*-coumaric acid derivatives were isolated and characterized from *A. calidophila* for the first time in this study. The previously isolated compounds (Viljoen *et al.*, 2001) showed strong antileishmanial activity (against *L. aethiopica* and *L. major*) and aloinoside was reported as better to arrest *L. aethiopica* and *L. major* activity than the standard drugs for Leishmaniasis (Abeje *et al.*, 2014). The isolation and characterization of such new compounds from *A. calidophila* and others coupled with the diversified and multipurpose use reports could attract further bioactivity research works. Similarly, the highly diversified compounds isolated from *Aloe harlana* and *Aloe pubescens* could have a factor for those diversified traditional values reported from these species. The highly diversified compounds could create better and complex synergetic effects to be more effective in remedial application (Cardarelli *et al.*, 2017). For example, microdontin A/B and aloin A/B isolated from *Aloe yavellana* showed larvicidal activity, however neither of the isolated compounds was as

active as the leaf exudate suggesting that the compounds acted synergistically or other unidentified bioactive compounds with potent larvicidal activity may exist in the leaf exudate (Tibebu *et al.*, 2018). The present study has revealed the presence of some more compounds in the leaf exudate of *Aloe yavellana*. Moreover, the unique compounds isolated and characterized from some *Aloe* species like, *Aloe pubescens*, *A. rivaie*, *A. secundiflora* and *A. tewoldei* could attract and instigate further studies for its biological activities, which has not been done so far.

Most of the *Aloe* species targeted in this study were found non-toxic to the higher dose level tested. However, some species like, *A. harlana*, *A. mcloughlinii*, and *A. pirottae*, which are known for wider and diverse therapeutic applications showed overt signs of behavioral changes and toxicity at the higher dose level with no mortality. Despite the growing popularity and presumed safety of herbal medicines, adverse effects have become a major safety issue for natural products (Han *et al.*, 2016). Consequently, in response to public health concerns, it is important to validate the safety of traditional herbal medicine before their use (El Kabbaoui *et al.*, 2017). The groups treated with exudates from *Aloe calidophila*, *A. harlana*, *A. mcloughlinii*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. pirottae*, *A. pubescens*, *A. rivaie*, *A. secundiflora*, *A. tewoldei*, *A. yavellana*, and unidentified/AHU53 were induced different levels of diarrhea but no sign of toxicity at both dose levels, which could be non-toxic at the level tested. An induced diarrhea could be due to the presence of the most common *Aloe* compound in these *Aloe* species known as aloin, which have laxative effect (Vanessa *et al.*, 2013; Cock, 2015). That is why the local communities used *Aloe* species like, *A. harlana*, *A. megalacantha* subsp. *megalacantha*, and *A. pirottae* as a colon cleaner. The different degree of diarrhea among the different groups treated with different exudates at same dose level could be due to the difference in aloin concentration (with equal dry weight of aloe exudates) among the *Aloe* species. The amount of aloin is extremely variable among different species and highly depends on the growing conditions (Viljoen *et al.*, 2001; Wamer *et al.*, 2003). The groups treated with exudates of *A. otallensis*, *A. rugosifolia* and *A. trichosantha* subsp. *longiflora* did not induced diarrhea, which could be indicated the absence of aloin. There were no any phytochemical studies reported aloin from *A. otallensis* and *A. rugosifolia* (Viljoen *et al.*, 1999; Biniam *et al.*, 2011). A single study so far done on the phytochemistry of an endemic species of *A. trichosantha* subsp. *longiflora* did not isolate aloin. Whereas, *Aloe harlana*, *A. megalacantha*

subsp. *megalantha*, and *A. pirottae* were induced high degree of diarrhea due to the higher concentration of aloin A and B in the leaf exudates of these species.

The significant difference ($P < .05$) in percentage change in mean absolute body weight and mean final body weight gain of *Aloe harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 at both dose levels could be attributed to potential toxicity. Significant level of body weight changes are an indicator of adverse side effects of test substances (Chitra *et al.*, 2015). The significant difference in percentage of mean relative liver weight for *Aloe harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 suggested potential toxic effect of these exudates at higher concentration, which might be fairly inflated the liver. In this respect, the wider traditional application of exudates from these *Aloe* species need to be given more safety attention for any treatment of human and livestock ailments. The exudate dose should be an important concern in the wider uses of *Aloe pirottae* and *A. harlana*. Because these two endemic *Aloe* species were known for diverse uses in the traditional medicinal uses (Anteneh *et al.*, 2012; Anteneh and Bussa, 2014). Although *A. megalacantha* subsp. *megalantha* and *A. megalacantha* subsp. *alticola* did not show any sign of toxicity the significance difference ($P > .05$) in their phytochemical profiles instigate taxonomic discussion on their current taxonomic position. This chemotaxonomic data supported that these sub species could be of two different species rather than sub species of *Aloe megalacantha*. Due to the complexities of the genus there are taxonomic problems encountered in the genus *Aloe* (Smith and Steyn, 2004; Grace *et al.*, 2013), and still taxonomic problems to be resolved are awaiting and new species are being discovered (Sebsebe and Gilbert, 2000), in which the chemotaxonomy will contribute.

6. GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1. Summary and Conclusion

The genus *Aloe* L. has long history as a multi-purpose herbal plant. In recent years, some *Aloe* species have gained significant interest among consumers and researchers due to their abundant secondary metabolites of medicinal and cosmetic values extracted from leaves as well as safety concerns due to potential toxicity. These increased interests in the phytochemical profile of *Aloe* species were due to the popularity of exudates as ingredient in pharmaceuticals and cosmetic formulations. Ethiopia, a recognized centre of diversity for *Aloe*, has 46 identified and documented species with three subspecies, of which 67.3% are endemic. Some research works done in Ethiopia indicated that the local communities make use of them for traditional medicines to treat health problems like, malaria (*A. pirottae* and *A. sinana*), tropical ulcer (*A. harlana*), and wound-healing (*A. sinana*). In addition, few species have been reported for potential toxicity when taken orally. This should be due to the presence of diverse and unique phytochemical compounds. Many traditional healers and users widely harvest *Aloe* species for medicinal and cosmetic purposes with the opinion that *Aloe* products are safe, however, this is not always the case with some *Aloe* species. Users of *Aloe* species for its medicinal and cosmetic value use the product with less precaution due to limited knowledge about the potential toxicity of few *Aloe* species. This calls the need for toxicity test of the *Aloe* species in Ethiopia due to the increasing interest on the products. Therefore, this PhD dissertation research has comprehensively analysed the existing multipurpose bio-cultural applications, bioactive compounds and potential toxic effects of leaf exudates from targeted endemic and near endemic aloes distributed in east and southeast of the Great Rift Valley Floristic regions of Ethiopia.

The comprehensive data on ethnomedicinal and bio-cultural values of *Aloe* species were studied through interview, focus group discussion (FGD), and guided field walks with 210 respondents. Then the relative frequency of citation (RFC_s), informants' consensus factor (F_{ic}), use value (UV_s), relative importance index (RI_s), and cultural value index (CV) were analyzed. The extraction, characterization, and quantification of phytochemical compounds were analysed from the leaf exudates of targeted *Aloe* species, which were subjected to a fully automated high-

performance thin layer chromatography (HPTLC), ultra-high pressure liquid chromatography–mass spectrometry (UHPLC-MS) coupled with ultraviolet-photodiode array detector (UV-DAD). Their potential toxicity have been analysed using oral acute toxicity test from leaf exudates of 17 *Aloe* species using Swiss albino mice (n=5/cage) following OECD 423 guide. A single oral dose of 300 and 2000mg/kg body weight at a rate of 10 mL/kg administered and major toxicity symptoms were monitored; and relative body and organs weights were measured daily until day 14.

Twenty-three *Aloe* species were recorded for ethno-medicinal and bio-cultural values with 196 use-reports and 2158 citations, grouped into six major use categories ($N_{UC}=6$). Medicinal use categories accounted the highest percentage for (76%). The species with the highest numbers of use-reports were *Aloe megalacantha* subsp. *alticola*, *A. trichosantha* subsp. *longiflora* and *A. calidophila* of which 87, 75 and 61.1% respectively were medicinal uses while *Aloe calidophila* has highest values in all indices. Among *Aloe* parts, leaf exudate accounted for 111 use-reports (49.1%) of which 92.9% were used for medicinal purposes. *Aloe retrospiciens* and *A. ruspoliana* were reported poisonous to carnivores. F_{ic} values of the six major use categories ranged from 0.86 to 0.22. Elderly people (>60) had more knowledge than 25-40 and 41-60 age groups (Kruskal-Wallis chi-squared=12.17, df=3, p=0.006), which is significant difference in depth of ethno-medicinal knowledge. Men had more knowledge of medicinal uses than women (Wilcoxon test, p=0.002) significantly different, while women were knowledgeable than men for cultural uses like, cosmetic (Wilcoxon test, p=0.06), not significantly different.

In phytochemical analysis with HPTLC method the solvent system containing ethyl acetate: acetonitrile: formic acid in the volume ratio of 9:2:1 v/v/v was found ideal mobile phase with 4% KOH in 96% MeOH derivatizer for Ethiopian *Aloe* species, which has been resulted in well-resolved bands of analyte compounds with distinct R_f values. The UHPLC-MS chromatogram of 14 *Aloe* species resulted in 56 peaks corresponds to compounds among which 35 (62.5%) were isolated and characterized. Anthrones and chromones were found dominant compound classes accounted 23 compounds (65.7%) among the isolated and characterized compounds. The most common compounds were aloin A/B from 11 species, aloinoside A/B from 11 species, and microdantin A/B isolated from eight species. In addition, a number of unique compounds like, 7-

O-methylaloesin, trihydroxy octadecenoic acid, 7-hydroxy-8-O-methylaloin B, deacetylaloin, 6'-O-coumaroyl aloesin, 7-O-methyl-2'-feruloylaloesin, 1-hexanol-pentosyl hexoside, 1-O-methylpicalaloside, isorabichromone, and caffeoyl ester of aloesin have been isolated and characterized for the first time from Ethiopian endemic *Aloe* species. The phytochemical characterization of *A. pirottae*, *A. megalacantha* subsp. *alticola*, *A. trichosantha* subsp. *longiflora* were done for the first time and resulted in unique characteristic compounds. In addition, the phytochemical variation was found significantly different ($P > .05$) between the two sub species of *Aloe megalacantha*. The isolation and characterization of bioactive phytochemical compounds were validated the existing traditional uses of aloes as well as the custodian of new compounds.

Oral acute toxicity test revealed that groups treated with exudates of *Aloe calidophila*, *A. trichosantha* subsp. *longiflora*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivaie*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei* and *A. yavellana* did not showed any signs of toxicity at both dose levels. The Median Lethal Oral Dose (LD_{50} -value) of these groups were estimated to be $>2000\text{mg/kg}$ body weight. Groups treated with *A. harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 showed overt signs of toxicity and significance difference ($P < .05$) of percentage change in body weight, body weight gain, relative liver weight, and food consumption compared to control group. *A. ruspoliana* and *A. retrospiciens* treatment groups showed severe level of toxicity signs and mortality of mice at both doses, LD_{50} -value was $>300\text{mg/kg}$ body weight.

In conclusion, the Ethiopian endemic and near endemic *Aloe* species were found to have multiple uses, but it has been shown that the most common local application was in the traditional health care system. More importantly, the acute oral toxicity test revealed the importance of safety concern while using *Aloe* species in traditional medicine since both non-toxic and potentially toxic *Aloe* species were resulted at the dose level tested in this study. In priority setting for *Aloe*-based product development and cultivation, species like, *A. calidophila*, *A. megalacantha* subsp. *alticola*, and *A. pirottae* were identified to be the top three ethno-medicinal and bio-culturally important endemic *Aloe* species. In addition, unidentified *Aloe* samples could instigate taxonomic investigation. The phytochemical profiles of *A. megalacantha* subsp. *megalacantha*

and *A. megalacantha* subsp. *alticola* showed more than 50% variation, which instigate taxonomic discussion on their current taxonomic position. This chemotaxonomic data supported that they could be of two different species rather than sub species of *Aloe megalacantha*. More importantly, the unique use-report: "poisonous to carnivores" from two *Aloe* species called *A. retrospiciens* and *A. ruspoliana* could initiate detailed phytochemical and toxicity studies, which have not been done so far. In this study, it has been shown that the leaf exudates were highly valued in medicinal applications. The higher number and unique compounds isolated and characterized from *Aloe* species targeted in this study might be contributed for the diverse medicinal values due to the synergetic effects of bioconstituents. Therefore, research interests on medicinal values of Ethiopian endemic aloes need to focus on exudates for phytochemical and pharmacological analysis. Because in this study, unique types of compounds were isolated and characterized from some *Aloe* species using UHPLC-MS-DAD methods, which were not reported so far for the Ethiopian *Aloe* species. In addition, the result showed the unfortunate decline in bio-cultural and ethnomedicinal knowledge between generations in most of the study areas. The deterioration of indigenous knowledge coupled with declining wild populations of most *Aloe* species could stimulate an urgent ethnobotanical study for in-depth investigation before it is lost. In general, the output of this comprehensive ethnomedicinal and bio-cultural knowledge will encourage the community to conserve, manage, and sustainable use *Aloe* species in the natural habitat as well as through cultivation.

Ethyl acetate: acetonitrile: formic acid in the volume ratio of 9:2:1 v/v/v was found ideal as mobile phase with 4% potassium hydroxide (KOH) in 96% methanol (MeOH) derivitizer in HPTLC studies for Ethiopian *Aloe* species, may be for east African *Aloe* species. Interesting to this work, double development, which has been carried out in automatic developer chamber followed with derivatization and scanning under UV 366 increased the visibility, optimized the band separation, and revealed well-resolved bands along all the tracks could be further applied.

The majority of these targeted *Aloe* species of Ethiopia were found good source of major anthrones, which is the most important class of compounds found in most aloes. Derivatives of anthrone are used in pharmacy as laxative, which stimulate the motion of the colon and reduce water reabsorption. This result is very important, since in vivo and in vitro studies have

demonstrated the laxative, antioxidant, antimicrobial, and anti-cancer properties of anthraquinones and anthrones. Among these anthrones, aloin was well known as a characteristic marker compound in most *Aloe* species and in this study aloin A and B showed relatively higher concentration in some species comparable to the concentrations and in few species were found significantly different ($P > .05$) to the well-known commercialized *Aloe* species. In addition, species like, *A. megalacantha* subsp. *megalacantha*, *A. pirottae*, *A. pubescens*, *A. secundiflora*, *A. trichosantha* subsp. *longiflora* and *A. mcloughlinii* could be considered as a good source of chromones such as aloeresins and aloesin. The higher concentration of plicataloside in *Aloe otallensis* and *A. rugosifolia*, nearly the dried exudate itself is a pure compound, which could be used as a standard without further processing.

The higher number and unique bioactive compounds isolated and characterized from *Aloe* species targeted in this study instigate further in vivo and in vitro tests promising for further studies of pharmacological activities. Some Ethiopian *Aloe* species like, *A. pirottae*, *A. secundiflora*, *A. tewoldei*, *A. mcloughlinii*, and *A. harlana* were found rich in compounds of commercial interest. In view of emerging trends of the *Aloe* products and enormous compounds isolated from aloes in this study, it was important to apply a comprehensive promotion of such potential *Aloe* species to attract the emerging cosmetic and pharmaceutical industries in Ethiopia with their wide scale cultivation and exploitation, in which the wild harvest is strictly prohibited by CITES. We hope that these findings will assist cosmetic and pharmaceutical industries in selecting specific Ethiopian *Aloe* species for wide-scale cultivation and resource exploitation in marginal and non-agricultural croplands suitable for *Aloe* cultivation.

More importantly, the two species, *Aloe retrospiciens* and *A. ruspoliana*, were found toxic at lower dose and need to be careful for the identity of wild harvested aloe products while using aloe exudate for any medicinal and cosmetic purposes. Nevertheless, further toxicological studies involving different mechanisms would be helpful to confirm the safety of these *Aloe* species in traditional medicine requires for safety concern due to an increasing public interest on aloe products in Ethiopia. To the best of our knowledge, this is the first *Aloe* toxicity experimental report from Ethiopian *Aloe* species, which could attract detail toxicological studies

for public safety reason in the current increasing interest of *Aloe* products in the Ethiopian markets from Ethiopian endemic *Aloe* species.

6.2. Recommendations

- The bioactivities and clinical trials of identified compounds need to be done based on the ethnomedicinal data reported in this study.
- There are a number of unidentified compounds in all species, which need to be determined.
- Two of the *Aloe* samples were unidentified at the species level, which could attract taxonomic analysis including molecular analysis for their identification and nomenclature.
- Molecular analysis is recommended to support the phytochemical data, which indicate significance difference in phytochemical profile of the two sub-species of *A. megalacantha*, which may lead them to be two different species of *Aloe* rather than ranked as sub-species.
- The need to use some endemic *Aloe* species for the production/preparation of chemical standards like *Aloe pirottae* having the highest number of compounds and identifying carrier molecules for Aloe exudates with pharmacological or cosmetic significance.
- The possibility to prepare standard for plicataloside from pure leaf exudate of *A. rugosifolia* and *A. otallensis* need to be considered.
- There are potentially toxic endemic *Aloe* species such as *Aloe retrospiciens* and *Aloe ruspoliana*, which required more attention and further sub-acute and chronic toxicity tests due to an increasing *Aloe* product market for safety concern.
- The two potentially toxic *Aloe* species, *A. retrospiciens* and *A. ruspoliana*, were not determined phytochemically in this study. Therefore, phytochemical studies with different protocol as well as taxonomic revision through molecular analysis are recommended.
- The diverse local varieties of *Aloe harlana* need further investigation.
- Additional studies to evaluate hepatotoxicity and nephrotoxicity should be carried out in order to provide further data on the less toxic *Aloe* species, like *A. harlana*, *A. pirottae*, *A. mcloughlinii* due to safety concerns on the reported medicinal uses.
- *Aloe* species reported as declining population in the wild from an endemic perspective and declining indigenous knowledge on their uses are pertinent to conservation concern.

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PAPER (ARTICLE) I

Ethnomedicinal and bio-cultural importance of aloes from south and east of the Great Rift Valley floristic regions of Ethiopia

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Research article

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ABSTRACT

There are 46 *Aloe* species identified from Ethiopia out of which 67.3% are endemics but comprehensive data on their ethno-medicinal and bio-cultural values are lacking. Interview, focus group discussion (FGD), and guided field walks were conducted with 210 respondents (152 men and 58 women). Relative frequency of citation (RFC_c), informants' consensus factor (F_{ic}), use value (UV_u), relative importance index (RI_i), and cultural value index (CV) were analyzed. Non-parametric Kruskal Wallis and Wilcoxon tests were performed using R software. Twenty-three *Aloe* species were recorded in the study areas with 196 use-reports and 2158 citations, grouped into six major use categories (N_{UC} = 6). Medicinal use categories accounted for 149 use-reports (76%) with 1607 citations. The species with the highest numbers of use-reports were *Aloe megalacantha* subsp. *alticola*, *A. trichosanthes* subsp. *longiflora* and *A. calidophila* of which 87, 75 and 61.1% respectively were medicinal uses. *Aloe calidophila* has highest values in all indices UV (11.72), RFC (0.68), RI (0.89), and CV (6.2). Among *Aloe* parts, leaf exudate accounted for 111 use-reports (49.1%) of which 92.9% were used for medicinal purposes. *Aloe retrospiciens* and *A. ruspiana* were reported poisonous to carnivores. F_{ic} values of the six major use categories ranged from 0.86 to 0.22. Elderly people (>60) had more knowledge than 25–40 and 41–60 age groups (Kruskal-Wallis chi-squared = 12.17, df = 3, p = 0.006), which is significant difference in depth of ethno-medicinal knowledge. Men had more knowledge of medicinal uses than women (Wilcoxon test, p = 0.002) significantly different, while women were knowledgeable than men for cultural uses like, cosmetic (Wilcoxon test, p = 0.06), not significantly different. The ways in which aloes are used and valued have implications for their future medicinal utility, which instigate detailed phytochemical and pharmacological studies.

1. Introduction

The genus *Aloe* L. belongs to family Asphodelaceae [1], which has 560 accepted species and 21 infraspecific taxa [2] is renowned for its use in herbal medicine throughout its native range in Africa South of the Sahara, the Arabian Peninsula, Madagascar and the Mascarene Islands [3]. The dried latex extracted from the leaves has been used medicinally in Africa, Asia, Europe and the Middle East for hundreds of years [4, 5, 6, 7, 8]. Aloes has been used for the treatment of wounds and skin complaints, malaria, microbial infections, and complaints of the digestive system [9, 10, 11, 12, 13]. In addition, commercial preparations containing *Aloe* species include laxative drugs, health drinks and tonics, after

shaving gel, mouthwash and toothpaste, hair tonic and shampoo, and skin-moistening gel [14, 15, 16].

Ethiopia, a likely centre of diversity for *Aloe*, has 46 identified and documented species with three subspecies, of which 67.3% are endemic. Several of these are highly threatened [17, 18]. Three local centres of endemism are recognised in Ethiopia, each with characteristic endemic species (Table 1) [18,19]. Six species of *Aloe* in Ethiopia were classified on the IUCN Red List [20] as endangered (*A. harlana* and *A. yavellana*), near threatened (*A. tewoldei* and *A. pubescens*), and vulnerable (*A. retrospiciens* and *A. rugosifolia*), while many of the remaining species are still data-deficient [21, 22, 23, 24]. Despite conservation concerns, aloes have been recognised for their economic potential in Ethiopia [19], particularly for livelihood security, economic development and enhancing biodiversity

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Table 1. The three local centres of *Aloe* endemism recognised in Ethiopia.

Center of endemism	Floristic regions	Number of endemic species	List of endemic species and infraspecific taxa
Northern and central highlands, north and west of the great rift valley	SU, KF, IL, WG, GJ, GD, WU, TU	15	<i>Aloe adigratana</i> , <i>A. ankoberensis</i> , <i>A. benishangulana</i> , <i>A. camperi</i> , <i>A. darkei</i> , <i>A. debrana</i> , <i>A. elegans</i> , <i>A. monticola</i> , <i>A. percrassa</i> , <i>A. pulcherrima</i> , <i>A. schelpei</i> , <i>A. sinana</i> , <i>A. steudneri</i> , <i>A. trigonantha</i> , and <i>A. weloensis</i>
Eastern highlands and lowlands	AF, HA	7	<i>Aloe bertemariae</i> , <i>A. harlana</i> , <i>A. mcloughlinii</i> , <i>A. megalacantha</i> subsp. <i>alticola</i> , <i>A. pirottae</i> , <i>A. pubescens</i> ; and <i>A. trichosantha</i> subsp. <i>longiflora</i>
Southern highlands, lowlands and rift valley	AR, BA, SD, GG	11	<i>Aloe elkerriana</i> , <i>A. friisii</i> , <i>A. ghibensis</i> , <i>A. gilbertii</i> subsp. <i>gilbertii</i> , <i>A. gilbertii</i> subsp. <i>megalacanthoides</i> , <i>A. jacksonii</i> , <i>A. kefaensis</i> , <i>A. otallensis</i> , <i>A. tewoldei</i> , <i>A. welmelensis</i> , and <i>A. yavellana</i>

SU = Shewa; KF = Kefa; IL = Ilubabur; WG = Welega; GJ = Gojam; GD = Gonder; WU = Welu; TU = Tigray; AF = Afar; HA = Hararge; AR = Arsi; BA = Bale; SD = Sidamo; GG = Gamo Gofa.

conservation on marginal lands [25]. For example, *Aloe debrana* leaf mesophyll is used in a thickening agent [26] and for treating sisal fibre for packing Ethiopian export coffee (e.g. www.gseventipic.com). In the Borena of Oromia region in Southeast Ethiopia, the local community has been cultivating *A. calidophila* to collect aloe leaf exudate and gel for small-scale aloe-soap manufacturing [27]. Data collated from the literature indicated that the medicinal uses of the genus *Aloe* comprised 74% of the use records [28] in which the use of exudate was common.

Despite the limited reports on the uses and conservation concerns for aloes of Ethiopia, their ethnomedicinal and biocultural values, and the impact of these values on sustainable use have not previously been assessed systematically. It is expected that people will be motivated to conserve resources that are most important to them, in contrast to resources perceived as less useful [29, 30, 31]. In this regard, further effort to improve the perception of local community towards the resource is needed. This can be achieved by indicating the invisible but potential values of the genus *Aloe* for effective conservation of the genus *Aloe*. In addition, it has been hypothesized that the local communities in all the study areas make use of *Aloe* species arbitrarily for similar purpose and

irrespective of different cultural/ethnic communities on the use of *Aloe* species. Therefore, we comprehensively investigated ethnomedicinal values, biocultural importance, and the emic perception of the wild population status of *Aloe* species in the East and South of the Great Rift Valley floristic region of Ethiopia.

2. Materials and methods

2.1. Study areas

This study focused on the eastern, southeastern, and southern floristic regions of Ethiopia called Hararge (HA), Bale (BA), Sidamo (SD), Arsi (AR), and Afar (AF) floristic regions, which are stated as east and south of the Great Rift Valley floristic regions in this study (Figure 1).

2.2. Data collection

Comprehensive data was collected on the ethno-medicinal and biocultural values, population trends of species in the natural habitat from

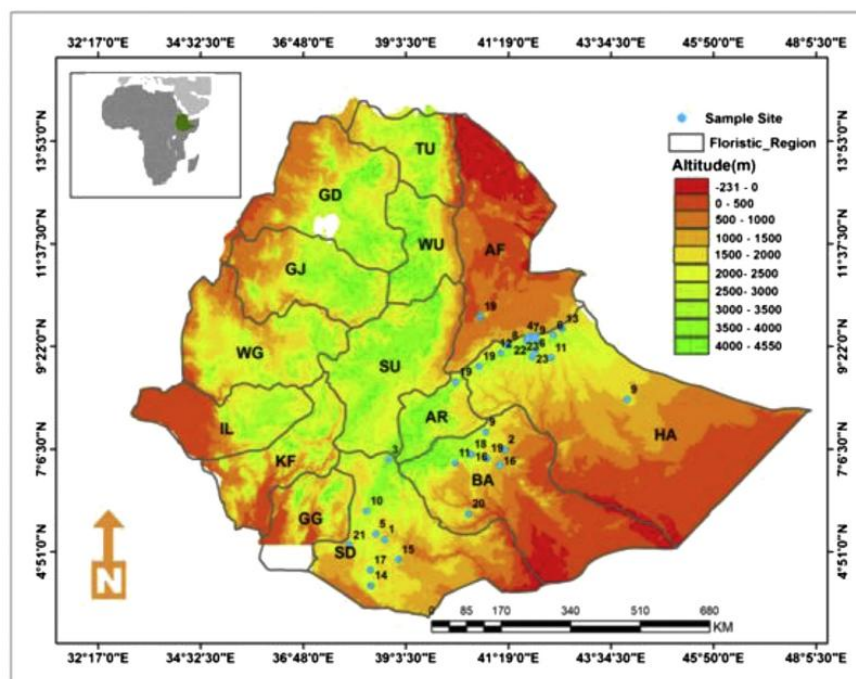


Figure 1. The study areas, stated as east and south of the Great Rift Valley floristic regions in this study (Site numbers indicated *Aloe* species: 1 = *A. calidophila*; 2 = *A. citrina*; 3 = *A. gilbertii* subsp. *gilbertii*; 4 = *A. harlana*; 5 = *A. lateritia*; 6 = *A. macrocarpa*; 7 = *A. mcloughlinii*; 8 = *A. megalacantha* subsp. *alticola*; 9 = *A. megalacantha* subsp. *megalacantha*; 10 = *A. otallensis*; 11 = *A. pirottae*; 12 = *A. pubescens*; 13 = *A. retrospectiens*; 14 = *A. rivae*; 15 = *A. rugosifolia*; 16 = *A. ruspoliana*; 17 = *A. secundiflora*; 18 = *A. tewoldei*; 19 = *A. trichosantha* subsp. *longiflora*; 20 = *A. welmelensis*; 21 = *A. yavellana*; 22 = AHU51; 23 = AHU53).

Table 2. Demographic characteristics of informants representing ethnic communities in east and south of the Great Rift Valley Floristic regions, Ethiopia.

Total Informant	Oromo (N = 140)	Somali (N = 32)	Afar (N = 24)	Harari (N = 14)
Men	97 (69.3%)	25 (78.1%)	19 (79.2%)	11 (78.6%)
Women	43 (30.7%)	7 (21.9%)	5 (20.8%)	3 (21.4%)
RI				
Men	60	16	14	8
Women	33	5	4	2
TH/key informants				
Men	37	9	5	3
Women	10	2	1	1
Age categories*				
25–40	50 (32.8 ± 5.3)	9 (31.1 ± 5.5)	4 (30.3 ± 4.0)	4 (32.6 ± 4.1)
41–60	65 (51.7 ± 5.8)	20 (50.3 ± 6.8)	15 (51.9 ± 5.9)	8 (51.1 ± 6.0)
above 60	25 (70.1 ± 6.4)	3 (76.0 ± 5.7)	5 (74.5 ± 6.6)	2 (72.0 ± 5.2)
Occupation				
Farmers	104	0	0	6
Agro-pastoralist	12	4	2	0
Pastoralist	18	26	22	0
Others	6	2	0	8

* Number of informants = N (mean age ± standard deviation) for each age categories; RI = random informants, TH = traditional healers.

the emic perspective, and associated indigenous knowledge of *Aloe* species in these floristic regions. Data was collected in the different seasons from 2017 (March to April and October to November) to 2018 (January to February and June to July). A total of 210 respondents (152 men and 58 women) from four cultural communities (Oromo, Somali, Afar, and Harari) participated. Informants were either randomly chosen (RI) or were systematically selected traditional healers (TH/key informants) who have in-depth traditional knowledge concerning multi-utility of *Aloe* species of their locality. Age, gender, and occupation (Table 2) were considered, as suggested by Martin [32] and Caruso et al. [33].

Ethnobotanical data collection procedures were approved by ethical committee for human involvement and use of lab animals in school of animal and range sciences of Haramaya University. Semi-structured interviews, focus group discussion (FGD), practical observation sessions, and guided field walk in *Aloe* localities were conducted after Oral Prior Informed Consent (PIC) was sought from every respondent. Most interviews were conducted in the field in order to avoid the risk of confusing identity of *Aloe* species. By repeated inquiries at least two times with the same informants the validity and reliability of recorded information was confirmed [32, 33, 34]. The respondents were asked to freely list all possible uses of each *Aloe* species, each time a plant was mentioned as “used” was considered as one “use-report”, and repeated mention of same use-report by different informants was taken as “use mention or number of citation”. Data recorded were: vernacular names of *Aloe* species, local uses, parts used, ingredients added during the use formulations (if any), and locally marketable *aloe* products produced. For medicinal use-reports, the ailments treated, preparation procedures, method of administration, and antidote (if any) were recorded. In addition, population trends (noticeably increasing, increasing, noticeably decreasing, decreasing, stable, and not sure/uncertain) from emic perspective were recorded (Appendix II). However, vague use-reports, from which it was difficult to distinguish the specific values were avoided, e.g. “used for livestock disease treatment”; “it has cultural importance”; etc. Finally, the use-reports recorded were categorized into major use categories (UC) of level 1 and sub-categories of level 2 using the Economic Botany Data Standard [35].

Aloe specimens were identified using taxonomic keys in the Flora of Ethiopia and Eritrea [17], through visual comparisons with authenticated plant specimens kept at the National Herbarium (ETH) of Addis Ababa University and at Herbarium of Haramaya University, and authenticated by Prof. Sebsebe Demissew (Professor of Plant Systematics

and Biodiversity). Voucher specimens of all species with all herbarium sheet data were deposited at both herbaria. Voucher numbers of Haramaya University were used in this study.

2.3. Data analysis

The data were organized and cleaned in an Excel spreadsheet (Microsoft office 2016), to be suitable for both qualitative and quantitative analysis. Qualitative data were analyzed following [32, 34, 36] like, free listing, part used, use-reports and use mentions. Quantitative data were analyzed using the corresponding formulas as follows:

2.3.1. Frequency of citation for use-report (FC_{UR})

Frequency of citation for use-report (FC_{UR}) is the percentage of informants who mentioned each use-report of particular *Aloe* species, which has been calculated using the formula:

$$FC_{UR}(\%) = n_i/N \times 100 \quad (1)$$

where n_i is the number of informants who cites each use-report per species and N is total number of informants.

2.3.2. Relative frequency of citation (RFC_s)

Relative frequency of citation was calculated by dividing the frequency of citation (FC) of a species by total number of informants (N) involved in the five floristic regions that makes N to varies or the summation of use-report ($\sum UR_i$) of all the informants interviewed for a species divided by N [37].

$$RFC_s = \frac{FC_s}{N} = \frac{\sum_{i=1}^n UR_i}{N} \quad (2)$$

2.3.3. Informant consensus factor (F_{ic})

The informant consensus factor (F_{ic}) of each *Aloe* species is the proportion of informants who independently reported its use against a particular use category calculated using the formula [38, 39]:

$$F_{ic} = n_{ur} - nt/n_{ur} - 1 \quad (3)$$

where, ‘ n_{ur} ’ is the “number of use-reports” in each use category and ‘ nt ’ is the “number of taxa used”.

2.3.4. Use value (UV)

Use value was used to demonstrate the relative importance of each *Aloe* species known locally, which can be calculated according to Albuquerque *et al.* formula [40]:

$$UV_s = \sum U_i / N_s \quad (4)$$

where, UV_s refers to the use value of *Aloe* species 's', U_i to the number of different uses mentioned by each informant i per specific *Aloe* species, and N is the total number of informants interviewed for *Aloe* species 's'.

2.3.5. Relative importance index (RI)

This index takes into account the number of major use-categories only and calculated as follows [37]:

$$RI_s = \frac{RFC_s(\max) + RNU_s(\max)}{2} \quad (5)$$

where, $RFC_s(\max)$ is the relative frequency of citation over the maximum. It is obtained by dividing FC_s by the maximum value in all *Aloe* species of the study [$RFC_s(\max) = FC_s / \max(FC)$], and $RNU_s(\max)$ is the relative number of use-categories over the maximum, obtained dividing the number of uses of the species $NU_s = u = u_{NC} u = u_i \sum UR_{ui}$ by the maximum value in all *Aloe* species of the survey $RNs(\max) = NUs / \max(NU)$.

2.3.6. Cultural value index (CV)

This index estimates the cultural significance of each *Aloe* species, which combines the three variables, informant (i), a species (s), and use-category (u), which is calculated using the following formula [41]:

$$CV_s = \left[NU_s / NC \right] \left[FC_s / N \right] \left[\sum_{i=1}^{u_{NC}} \sum_{i=1}^{i_u} UR_{ui} / N \right] \quad (6)$$

The first factor is the relationship between the numbers of different uses reported for ethnospecies (each *Aloe* species) and total number of use-categories. The second factor is the relative frequency of citation of a species. The third factor is the sum of all the UR for a species, i.e., the sum of number of participants who mentioned each use of a species, divided by N .

To test if there was any correlation between age of the informant and their knowledge on use of aloes (number of use-reports), the nonparametric Kruskal Wallis Test was performed. If there was a significant difference between the informant's gender and knowledge about use of aloes, the non-parametric pair wise Wilcoxon test was performed using R software version 3.3.4. for Windows using multcompview and R companion packages. P-values of less than 0.05 were taken as statistically significant difference.

3. Results

3.1. Ethno-medicinal and bio-cultural values of aloes with frequency of citation

A total of 23 *Aloe* species (Appendix I) were recorded in the study areas, of which 21 are found in the Flora of Ethiopia and Eritrea [17] and two (recorded as unknown, voucher number AHU51 and AHU53) could not be identified to species level. Among the *Aloe* species reported in this study, 11 species (52%) are endemic and near endemic. The total number of use-reports was 196, were categorized into six major use categories ($N_{UC} = 6$) with 2158 citations (use mentions) from the 23 *Aloe* species (Table 3) by 210 respondents (Table 1). The major use categories (UC) are medicines (Md), social uses (SU), materials (Mt), environmental uses (EU), vertebrate poisons (VP), and food (Fd). The medicinal use category accounted 149 use-reports (76%) with 1607 citations. The highest

number of use-reports was recorded for *Aloe megalacantha* subsp. *alticola* with 23 use-reports, of which about 87 % were medicinal uses for humans and livestock. The next most frequently cited species was *A. trichosantha* subsp. *longiflora* with 20 use-reports followed with *A. calidophila* with 18 use-reports, in which 75% and 61.1% were medicinal (human and veterinary) uses, respectively (Table 3).

The highest frequency of citation was 100% recorded for *Aloe otallensis* leaf exudate used for weaning children from breast-feeding followed with 94.4% for *A. yavellana* leaf exudate used to treat jaundice, 91.6% for *A. rivae* leaf gel used for body and hair wash, and 90.9% for *A. megalacantha* subsp. *alticola* exudate to treat skin infections (Table 3).

The most frequently cited use of *Aloe* species in the study areas was for medicines (human and veterinary): 149 use-reports (76%) from 22 *Aloe* species, with a total number of 1607 citations (use mentions) were recorded. The least use-report was for food use-category: three use-reports (1.5%) from three *Aloe* species with 19 citations (Figure 2).

A total of nine different plant parts were used in the diverse bio-cultural uses (Figure 3) within the six major use categories. Leaf exudate was the most frequently sought part, accounting for 111 use-reports (49.1%) in which about 89 (80.2%) were for human medicinal formulations and 12 (11.7%) were for livestock medicinal formulations. That means, 92.9% of exudate were used for medicinal purposes and the remainder in formulations were in social uses and vertebrate poisons categories. In addition, the entire leaf for 47 use-reports (20.8%), which also include the use of exudate as part of entire leaf. This would suggest that the use of exudate exceeds 69% of the plant parts used for medicinal purpose. The fewest use-reports were reported from formulations made from the inflorescence and pedicel, with just a single use-report, which is 0.4% each (Figure 3).

The local communities practiced nine types of preparation methods for medicinal applications, out of these 42% are prepared in the form of pure exudate collection from the fresh leaf to be used for different application followed by pulverization (11.5%) and concoction (10.8%) (Figure 4).

3.2. Use-reports among cultural communities

The distribution of use-reports among the cultural communities in the study areas showed 161 use-reports for the Oromo community ($N = 140$), followed with 17 for the Somali community ($N = 32$), 9 for the Afar community ($N = 24$), and 9 use-reports for the Harari community ($N = 14$). In most of the study areas, the medicinal uses of *Aloe* species were found more popular among the Oromo community, which accounted for 125 medicinal use-reports, in which 77.6% of the total use-report of this community. The fewest uses were reported from the Afar and Harari communities with 9 use-reports each. Among the bio-cultural values, environmental use-reports like boundary marking, soil conservation, and living fence support were documented only from the Oromo community, from four *Aloe* species. A unique use-report "poisonous to carnivores" was reported by the Somali and Oromo communities for two *Aloe* species called *Aloe retrospiciens* Reynolds & Bally and *A. ruspoliana* Baker, respectively.

The Oromo community used 22 out of the 23 species documented in this study (the exception was *A. retrospiciens*). The Somali community used four species (*A. retrospiciens*, *A. megalacantha* subs. *megalacantha*, *A. mcloughlinii*, and *A. pirottaei*) followed by the Afar community who use two species, *A. retrospiciens* and *A. trichosantha* subsp. *longiflora* and the Harari community who also use two species, *A. trichosantha* subsp. *longiflora* and *A. macrocarpa*.

3.3. Ethno-medicinal and bio-cultural knowledge among gender and age categories

The level of knowledge of *Aloe* species diversity and use-reports are noticeably lower in the younger age categories compared to elderly

Table 3. List of *Aloe* species, major use categories, and frequency of citation per use reports (FC). E = Exudate; F = Flower; G = Gel; I = Inflorescence; L = Leaf; LP = Live plant; P = Pedicel; R = Root.

Scientific name & Voucher No.	Major use category	Sub-category	Use report	Part used	Use description	FC%	
<i>Aloe caldophila</i>	Social uses	Memorial	Memorial	LP	Planting on graveyard	94.44	
Reynolds		Weaning	Weaning child from breastfeeding	E	Apply to the nipple/breast	88.89	
AHU103	Medicines	Magic	Belief to increase livestock herd size	LP	Planting at gate of the traditional cattle shelter	61.11	
		Cosmetics	Skin softening	G	Apply on skin as a balm	22.22	
		Endocrine system	Bile duct problem/Jaundice	R	Pulverize with honey & drink	83.33	
				P	Chew & swallow the fluid	27.78	
		Infections and infestations	Sexually transmitted infections/STI	L	Smoke-bathe the genitals	77.78	
				E	Fresh exudate taken orally	44.44	
			Malaria	R	Pulverize with water & drink filtrate	44.44	
				E	Drop in infected eye	44.44	
			Eye infection	E	Put on the head & apply externally around infected eye	27.78	
				L	Smoke-bathe the genitals	38.89	
			Gonorrhea	E	Apply externally all around the eye	27.78	
				E	Apply externally	72.22	
		Skin and subcutaneous tissue	Wound healing	E	Apply externally	72.22	
				L	Warm fresh leaf & keep on small swellings	61.11	
			Small swelling on skin locally called <i>soochia</i>	L	Warm fresh leaf & keep on small swellings	61.11	
				Wound of livestock due to carnivore/hyena attack	L	Fresh leaf crushed and tied on wound & smoke-bathed	55.56
	Musculo-skeletal system	Bone pain	L	Warm well & keep on painful part repeatedly	61.11		
			Bone pain of cattle	L	Warm well & keep on painful part repeatedly	61.11	
	Materials	Domestic utensils	Repel flies from wounds	E	Apply externally	38.89	
		Soap making	Soap making	G	Used in small-scale soap production with ingredients	22.22	
	Food	Metabolic system	Water source	G	Fresh gel eaten as source of water in extremely hot areas	33.33	
<i>Aloe citrina</i> Carter & Brandham	Medicines	Skin and subcutaneous tissue	Wound healing	E	Apply externally	75.00	
			Leg and hand swelling	G	Tie onto swollen part	62.50	
AHU123	Medicines	Infections and infestations	Wound healing in livestock	E	Apply externally	75.00	
			Eye infection	E	Drop in infected eye	75.00	
		Malaria	E	Taken orally	50.00		
			E	Taken orally	75.00		
		Digestive system	Abdominal disorder	E	Taken orally	75.00	
		Endocrine system	Bile duct problem/Jaundice	E	Taken orally	91.67	
	Digestive system		Colon cleaner	E	Taken orally	75.00	
<i>Aloe giberitii</i> Reynolds ex Sebsebe & Brandham subsp. <i>giberitii</i>	Medicines	Gastric	L	Fresh young leaf pulverized & filtrate take orally	58.33		
			L	Fresh young leaf pulverized & filtrate given orally	41.67		
		Skin and subcutaneous tissue	Wound healing	E	Apply externally	75.00	
			Infections and infestations	Eye infection	E	Drop in infected eye	58.33
AHU102		Medicines	Infections and infestations	Eye infection	E	Drop in infected eye	58.33
				Malaria	L & E	Concocted & taken orally	25.00
	Environmental uses	Barrier	LP	Planting	75.00		
			Soil improver	Soil conservation	LP	Planting on terracing	66.67
	Social uses	Memorial	Memorial	LP	Planting on graveyard	58.33	
<i>Aloe harlana</i> Reynolds	Medicines	Skin and subcutaneous tissue	Wound	E	Apply on wound	83.33	
AHU117			Skin inflammation	G & E	Used as ointment	27.78	
		Snake and spider bites	Snakebite	L	Pulverized with water & filtrate taken orally	72.22	
			Infections and infestations	Hair fungus	G	Apply on hair or wash with fresh gel daily	61.11
		Skin fungus	Used as ointment	G & E	Used as ointment	50.00	
			Digestive system	Bloated stomach of calves locally called <i>shizho</i>	L	Fresh leaf pulverized & filtrate given orally	77.78
		Colon cleaner	E	Powder (locally called <i>SIBRI</i>) taken orally in water	27.78		
			Endocrine system	Liver swelling	L	Pulverized and filtrate taken orally	22.22
		Spleen swelling/Splenomegaly		L	Pulverized and filtrate taken orally	22.22	
	Materials	Domestic utensils	Repel honeybees during honey harvest	L	Smoked while harvesting the honey to prevent bee stings	38.89	
<i>Aloe lateritia</i> Engler	Social uses	Cosmetics	Hair wash	G	Make shampoo for hair wash	66.67	
AHU125			Soften hard skin	G	Scrape the gel & apply on skin	41.67	
	Medicines	Skin and subcutaneous tissue	Skin infection	G & E	Apply on skin	58.33	
			Infections and infestations	Eye infection	E	Drop in the eye	58.33
<i>Aloe macrocarpa</i> Todaro	Social uses	Cosmetics	Emollient	G	Scrape the gel & apply on skin	65.63	
AHU19	Medicines	Infections and infestations	Skin diseases/ fungal	G & E	Apply on skin	59.38	
			infestations	Hair fungus	G	Apply on hair or wash with fresh gel	50.00
		Eye infection	E	Drop in eye	40.63		
			Infections and infestations	Malaria	E	Collect & drink	12.50
	Skin and subcutaneous tissue	Fire burn		G	Apply immediately during an accident	37.50	
			Wound healing	E	Apply on wound as a cream	28.13	
		Wound healing of livestock	E	Apply on wound as a cream	31.25		
			Reproductive system and sex health	Sexual impotency	R	Pulverized, mix with fresh butter and use as ointment & smoke-bathe the penis	9.38
<i>Aloe mcloughlinii</i> Chris.	Medicines	Skin and subcutaneous tissue	Wound healing/ goats	E	Powdered and applied on wound	62.50	
		subcutaneous tissue	Wound healing/ goats	E	Apply on infected part	50.00	

Table 3 (continued)

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Table 3 (continued)

Scientific name & Voucher No.	Major use category	Sub-category	Use report	Part used	Use description	FC%
		Endocrine system	Liver disease	E	Taken orally	31.82
		Infections and infestations	Anthrax	L, F & R	Concocted & given orally	13.64
	Social uses	Memorial	Graveyard	LP	Planting on graveyards	72.73
	Environment	Boundaries	Fence support	LP	Planting	68.18
	al uses	Soil improver	Soil conservation	LP	Planting on mountain slopes along the terrace	36.36
	Food	Food	Food	G	Fresh gel scraped and eaten	31.82
^a <i>Aloe retrospiciens</i> Reynolds & Bally	Vertebrate poisons	Poison	Poison carnivore	E	Concentrated exudate hide in meat to feed hyena	58.33
AHU160		Poison	Kill goats if eaten in dry season, due to starvation	L	If eaten in the drought season can kill goats	41.67
		Poison	Poison rats	E	Dried exudate and apply in rat feed	25.00
<i>Aloe rivae</i> Baker	Social uses	Cosmetics	Body & hair wash	G	Make shampoo for washing	91.67
AHU115	Medicines	Snake and spider bites	Snakebite	E	Taken orally	75.00
		Skin and subcutaneous tissue	Wound healing	E	Apply externally	66.67
		Cancer	Breast cancer	E	Taken orally	25.00
	Materials	Domestic utensils	Repel flies	E	Apply externally on wound	41.67
^a <i>Aloe rugosifolia</i> Gilbert & Sebsebe	Medicines	Musculo-skeletal system	Bone pain	L	Warmth well & keep on painful part repeatedly	77.78
AHU113		Skin and subcutaneous tissue	Wound healing	E	Apply externally	72.22
			Wound of livestock	E	Apply externally	61.11
			Small swelling on skin locally called <i>socsa</i> , on hand and legs	L	Warm & keep on small swellings	38.89
		Infections and infestations	Eye infection	E	Drop in infected eye	44.44
<i>Aloe ruspoliana</i> Baker	Vertebrate poisons	Carnivor prevention	Deter carnivore from night shelter of livestock	LP	Planting around livestock night shelter	87.50
AHU121		Rodent control	Deter rats due to bad smell	LP	Planting around rats nests	50.00
	Medicines	Skin and subcutaneous tissue	Itching skin on goat locally called <i>cimo</i>	L	Warm & rub the skin while warmer	37.50
<i>Aloe secundiflora</i>	Medicines	Skin and subcutaneous tissue	Skin infections	E	External use on skin	88.89
Engler			Wound healing	E	Drop on wound and bandage	83.33
AHU106			Skin infection of livestock	L & E	Prepared for external use on skin	77.78
			Wound on livestock skin	L & E	Prepared & applied to wound	61.11
		Musculo-skeletal system	Inflammation in muscles/Rheumatism	G & E	Mix & tie on inflamed part	38.89
		Infections and infestations	Ectoparasite	L & E	Concocted for external use on skin	50.00
			Malaria	E	Taken orally	33.33
			Diarrhea	E	Taken orally	22.22
^a <i>Aloe teweldei</i> Gilbert & Sebsebe	Medicines	Musculo-skeletal system	Bone fracture	G	Bandage the gel around to soften the part before traditional fracture medication	87.50
AHU120		General Ailments with Unspecific Symptoms	Cold problem	L	Pulverized & filtrate taken orally	87.50
		Digestive system	Stomach disorder	L	Pulverized & filtrate taken orally	87.50
		Skin and subcutaneous tissue	Itching skin on goat locally called <i>cimo</i>	L	Put in fire until very soft then rub on skin	62.50
	Social uses	Poison	Crop pest control	E	Powder used in crop storage	50.00
^a <i>Aloe trichosantha</i> subsp. <i>longiflora</i> Gilbert & Sebsebe	Medicines	Digestive system	Laxative/Purgative, colon cleaner, Constipation	E	Powdered & drink in the form of solution or swallow with banana	50.98
		Skin and subcutaneous tissue	Infection on skin or wound/Antidermatosis	E	Apply on infected skin	33.33
AHU05			Fire burn	G	Applied externally	9.80
			Skin hardening/emollient	G & E	Make juicy & apply on skin	4.90
		Snake and spider bites	Snakebites antidote	L & E	Concocted with water & taken oral	23.53
		Endocrine system	Bile duct problem	L	Pulverized kept for 12 h and drink filtrate/young leaf	22.55
		Infections and	Tonsillitis	E	Drops on the throat	11.76
		infestations	Malaria	E	Taken orally	10.78
				L	Pulverize & filtrate taken orally	1.96
			Eye infection	E	Drop in infected eye	6.86
		Skin and subcutaneous tissue	After male circumcision	L	Smoke bathed after circumcision to prevent potential infection	14.71
			Wound on livestock skin	E	Apply externally on wound	14.71
		General Ailments with Unspecific Symptoms	Pain due to cold	G	Massage the pain part softly	10.78
		Sensory system	Improve poor sight	E	A drop in eyes	0.98
		Reproductive system and sex health	Infertility of man and woman	G	Wash the body and genitalia	0.98
		Pregnancy, birth and puerperial	Delayed placenta in cattles	L & R	Concoction given orally	5.88
	Social uses	Weaning	Weaning child from breastfeeding	E	Apply to the nipple/breast	50.00
		Magic	Increase herd size of livestock and camel	L	Smoke-bathe milking utensils	1.96
		Illuminant	Lighting bonfire	I	Lighting bonfire/torch with dried sticks used in Christian holidays	6.86
	Materials	Domestic utensils	Repel flies from wounds	E	Apply on and around the wound	18.63
	Food	Metabolic system	Relief dehydration in extreme hot condition	G	Make it free from exudate & eaten	5.88
^a <i>Aloe welmelensis</i> Sebsebe & Nordal	Medicines	Skin and subcutaneous tissue	Wound healing	E	Apply externally	75.00
AHU124			Wound healing for cattles	E	Apply externally	75.00
^a <i>Aloe yavellana</i>	Social uses	Weaning	Weaning child from breastfeeding	E	Apply to the nipple/breast	94.44
Reynolds	Environment al uses	Soil improver	Soil conservation	LP	Planting on terracing	88.89
AHU116		Boundaries	Fence support	LP	Planting	61.11
	Medicines	Skin and subcutaneous tissue	Wound healing	E	Apply externally	72.22

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Table 3 (continued)

Scientific name & Voucher No.	Major use category	Sub-category	Use report	Part used	Use description	FC%
		Skin and subcutaneous tissue	Wound healing of livestock	E	Apply externally	72.22
		Ectoparasite of livestock		L & E	Concocted for external use on skin	50.00
		Snake and spider bites	Snake poison	E	Taken orally	44.44
	Materials	Domestic utensils	Mosquito repellent	L	Smoking around to stifle mosquito	66.67
'Highly spotted aloe'	Medicines	Snake and spider bites	Snake poison	E	Drink very soon after snakebite	63.33
AHU53		Spider poison		E	Drink very soon after spiderbite	56.67
		Skin and subcutaneous tissue	Wound healing	E	Powder apply on wound	56.67
		Digestive system	Diarrhea in cattles	L	Pulverized & filtrate given orally	46.67
	Materials	Pest control	Crop pest	E	Powdered & applied in traditional crop storage	36.67
Unidentified	Medicines	Infections and	Hair fungus	G	Apply on hair	83.33
AHU51		infestations	Tonsillitis	E	Drop in the throat	61.11
		Endocrine system	Diabetics	E	Powder in solution & drink daily morning	72.22
	Social uses	Cosmetics	Skin softening	G	Apply on skin	66.67

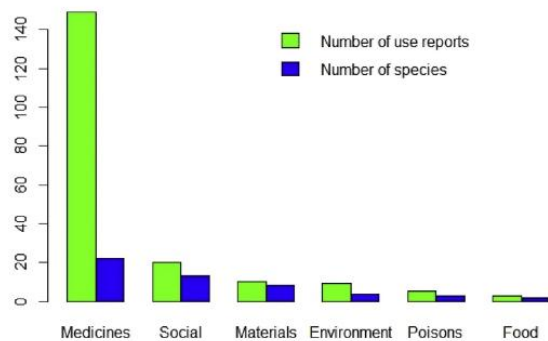
* Endemic.

* Narrowly endemic.

□ Endangered.

◇ Near threatened.

** Vulnerable.

Figure 2. The number of use-reports and number of *Aloe* species used in each major use category.

informants. For example, out of the total citations (1336) for human medicinal uses, 654 were by elderly people (above 60 years, N = 35) followed with 398 use mention by adults (age 41–60, N = 108) and 284 by young informants (age 25–40, N = 67). The highest number of use-report in all major use categories such as medicines (Md), social uses (SU), materials (Mt), environmental uses (EU), vertebrate poisons (VP), and food (Fd) were reported by elderly people (Figure 5). In the medicines category, 21 species were reported by elderly people (above 60 years, N = 35) followed by 16 species by adults (age 41–60, N =

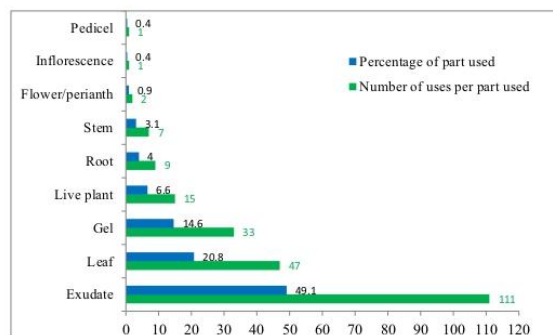


Figure 3. Number of uses and percentage of each part used for the respective treatment purposes.

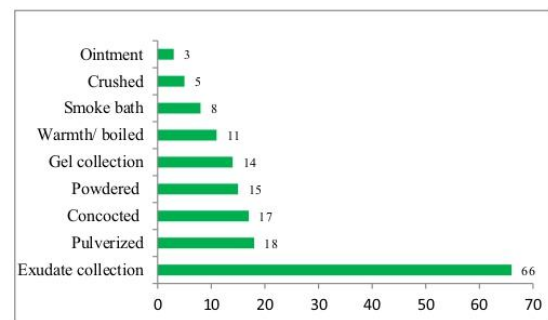


Figure 4. Number of preparation in each type of preparation method for medicinal uses.

108) and eight species by young informants (age 25–40, N = 67) (Figure 6).

The depth of comprehensive bio-cultural knowledge among different age groups indicated that elderly people (above 60 years) had a much deeper knowledge than the two age group ranging from 25 to 40 and 41–60 (Kruskal-Wallis chi-squared = 12.17, df = 3, p = 0.006*). There is a significant difference in the depth of ethno-medicinal knowledge

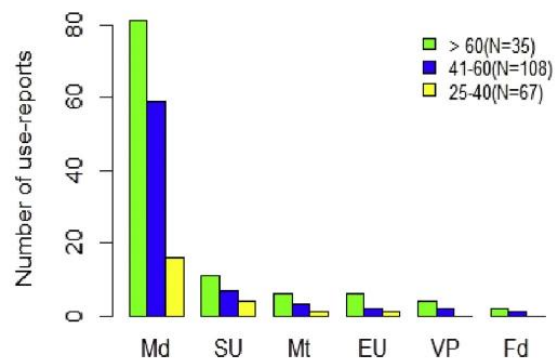


Figure 5. Number of use-reports in each major use category for each age group (medicines (Md), social uses (SU), materials (Mt), environmental uses (EU), vertebrate poisons (VP), and food (Fd)).

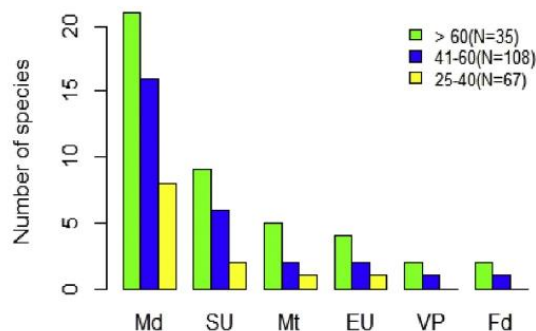


Figure 6. The number of *Aloe* species reported in each major use category for each age group (Md), social uses (SU), materials (Mt), environmental uses (EU), vertebrate poisons (VP), and food (Fd).

between the 25–40 age group and the above 60 age group ($p = 0.0004^{**}$), and a significant difference between 41–60 year olds and the above 60 age group ($p = 0.02^{*}$) ($p < 0.05$). It was observed that many young people in the study areas were found less knowledgeable about the different *Aloe* species and associated medicinal and bio-cultural values. In addition, men are more knowledgeable on identifying *Aloe* at the lower taxonomic level and diverse medicinal use-reports than women. The men from all communities had deeper knowledge in the medicinal use category than women (Wilcoxon test, $p = 0.002$), which is significantly different ($p < 0.05$). Women were found more knowledgeable than men for cultural uses like, cosmetic (Wilcoxon test, $p = 0.06$), not significantly different ($p > 0.05$).

3.4. Informant consensus factor and value indexes

Informant consensus factors (F_{ic}) of the seven major use categories ranged from 0.86 to 0.22. The highest F_{ic} value 0.86 was for medicines with 22 species and 11 use sub-categories, followed by 0.63 for environmental uses with four species and three sub-categories, and least F_{ic} value was 0.22 for materials with eight species and two sub-categories (Table 4).

Aloe species were compared based on the values of importance metrics. Some species like *Aloe calidophila*, *A. megalacantha* subs. *alticola*, *A. pirottae*, and *A. gilbertii* subsp. *gilbertii* showed higher values across the indices due to be mentioned by a higher number of informants, relatively higher number of use-categories, and larger number of use-reports to elucidate species that were of high importance in all indices (Table 5).

4. Discussion

4.1. Ethno-medicinal and bio-cultural values of aloes

Most of the *Aloe* species were reported to have multiple uses, but the majority of uses were medicinal [42, 43]. In this study, it has been shown

that most common traditional use of *Aloe* species was in the traditional health care system. This compares closely with the value of 74% of literature-based use records describing the medicinal uses of *Aloe* [28] and 73% for human medicines from 11 *Aloe* species in Tanzania [43]. There are 12 sub-categories under the medicine (Md) major use category like the most cited applications of *Aloe* species were for skin and subcutaneous tissue ailments accounted for 29.6%, followed with infections and infestations 26.5%, and digestive system 14.5%. *Aloe* exudates was the most cited *Aloe* part used in the medicinal uses deserve further investigation into the phytochemistry and pharmacological activities. The beneficial health-promoting properties present in the exudates of *Aloe* leaves are attributed to their diverse phytochemical composition [44]. Phytochemical studies on the genus *Aloe* indicated that over 200 compounds belonging to different classes such as anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes, anthraquinones and flavonoids have so far been reported [44, 45, 46, 47]. These classes of compounds have been shown to possess antiviral, anti-tumor, and antibacterial activities [10, 13, 28, 42]. Numerous *in vitro* and *in vivo* pharmacological and clinical trials have been revealed the traditional uses of *Aloe* including wound healing, anti-ulcer, anti-diabetic, hypoglycaemic, anti-cancer, anti-bacterial, anti-viral, and anti-hyperlipidemic activities [10, 42, 48].

A unique use-report: "poisonous to carnivores" was reported for two *Aloe* species: *Aloe retrospiciens* and *A. ruspoliana*. This study is the second to sample *A. retrospiciens*, 60 years after the first collection of a type specimen for this species. There was no any data on this species except that it was listed as vulnerable (VU) in IUCN category with an unknown population trend [22]. Meat painted with *A. ruspoliana* is used as bait to kill hyenas [49]. Similarly, *Aloe buettneri*, *A. lateritia*, *A. rabaiensis*, *A. secundiflora*, and *A. zebrina* have been documented as ingredients in arrow poisons throughout Africa [50]. The leaf exudates of these two species have an unpleasant smell and found free from the most common Aloes compound called aloin using TLC profile using pure aloin as standard.

The higher frequency of citations of some use-reports can be explained by the fact that these *Aloe* species are best known and have long been used by the majority of informants, representing a source of reliability. For example, the highest FC value for *A. otalensis* used for weaning breast feeding child could be attributed for the more effectiveness of its exudate, which is much bitter than others. Similarly, the local communities in Kenya claimed that *Aloe lateritia* is not as bitter as *A. secundiflora*, and hence not as effective [7] so the community has preference of specific *Aloe* species for the purpose.

Among the 23 *Aloe* species reported in this study, five species were reported whose gel is used for cosmetic purposes: *Aloe calidophila*, *A. lateritia*, *A. macrocarpa*, *A. rivae*, and the unidentified/AHU 51. The cosmetic value reported with higher FC for *A. rivae* makes this species a good candidate for detailed study of its gel. Taxonomic reports have indicated that *A. lateritia* and *A. macrocarpa* are grouped in the Saponaria [19] or maculate group, a name that came from the Latin "sapo" meaning soap, as the gel makes a soapy lather in water. The gel of AHU51 is used for cosmetic purposes, which has been observed while making soapy

Table 4. Major use categories with the corresponding sub-categories, number of *Aloe* species used and informant consensus factor values.

Major use categories with sub-categories	No. of sub-category	N. of use reports	No. of citation	No. of species	F_{ic}
Medicines: Blood and cardiovascular system, cancer, digestive system, infections and infestations, general ailments with unspecific symptoms, endocrine system, snake and spider bites, pregnancy, birth and puerperal, reproductive system and sex health, sensory system, skin and subcutaneous tissue, & musculo-skeletal system	12	146 (76%)	1607	22	0.86
Social uses: Weaning, illuminant, magic, memorial, tattoo & cosmetics	7	20 (10.2%)	276	13	0.37
Materials: Domestic utensils & soap making	2	10 (5.1%)	107	8	0.22
Environmental uses: Soil improver, boundaries, & barrier	3	9 (4.6%)	112	4	0.63
Vertebrate Poisons: Poison, carnivore prevention, rodent control, & pest control	6	5 (2.6%)	37	3	0.50
Food: Metabolic system & edible	2	3 (1.5%)	19	2	0.50

Table 5. Relative Importance metrics of *Aloe* species used in the Great Rift Valley floristic regions of Ethiopia.

Species	UC	UR	UV	RFC	RI	CV
<i>Aloe calidophila</i>	4	18	11.72	0.68	0.89	6.20
<i>Aloe megalacantha</i> subsp. <i>alticola</i>	3	23	10.89	0.52	0.88	5.66
<i>Aloe pirottae</i>	3	14	6.92	0.49	0.66	2.06
<i>Aloe gilbertii</i> subsp. <i>gilbertii</i>	3	10	6.00	0.60	0.66	1.55
<i>Aloe pubescens</i>	4	12	5.86	0.49	0.62	1.49
<i>Aloe megalacantha</i> subsp. <i>megalacantha</i>	2	9	5.63	0.63	0.66	1.39
<i>Aloe yavellana</i>	4	8	5.00	0.63	0.63	1.10
<i>Aloe harlana</i>	2	10	4.83	0.48	0.57	1.01
<i>Aloe secundiflora</i>	1	8	4.55	0.57	0.59	0.90
<i>Aloe trichosantha</i> subsp. <i>longiflora</i>	4	20	3.14	0.24	0.61	0.64
<i>Aloe otallensis</i>	2	7	4.92	0.61	0.60	0.91
<i>Aloe macrocarpa</i>	2	9	3.44	0.55	0.60	0.74
<i>Aloe rivae</i>	3	5	3.91	0.58	0.53	0.49
<i>Aloe citrina</i>	1	6	4.12	0.51	0.51	0.55
<i>Aloe rugosifolia</i>	1	5	2.94	0.39	0.39	0.25
Unidentified (AHU51)	2	4	2.83	0.61	0.54	0.30
Unidentified (AHU53)	2	5	2.60	0.52	0.49	0.28
<i>Aloe tewoldei</i>	2	5	2.50	0.33	0.34	0.18
<i>Aloe mcloughlinii</i>	1	6	2.42	0.40	0.43	0.25
<i>Aloe lateritia</i>	2	4	2.25	0.56	0.50	0.22
<i>Aloe welmelensis</i>	1	2	1.50	0.38	0.32	0.12
<i>Aloe retrospiciens</i>	1	3	1.25	0.42	0.37	0.07
<i>Aloe ruspoliana</i>	2	3	1.17	0.28	0.27	0.05

UC = number of major use categories per species; UR = number of use-reports per species; UV = use value of a species; RFC = relative frequency of citation for species; RI = relative importance index; CV = cultural value index of species, which considered the three factors i.e. s (species), i (informants), and u (uses).

lather in water. Though *A. calidophila* is not in the Saponaria group, its gel is used for soap making and in few sites of the study areas two women associations were observed cultivating this species and used in small-scale soap production for local market, branded as "Yoya" meaning 'peace' in Borena and labelled to treat skin fungus and infections. It has been reported that the gel of *Aloe* species exhibited faster wound healing effects [48].

4.2. Use-reports among the cultural communities

People of the five floristic regions use 23 species of *Aloe*, and the diversity in uses suggests that species are not used interchangeably. The highest number of use-reports, recorded for *A. calidophila*, *A. megalacantha* subsp. *megalacantha*, *A. trichosantha* subsp. *longiflora*, and *A. pirottae* could be associated with the wider geographic distribution of species and their use by different ethnic communities, a pattern that has been noted previously in Kenya [7]. The higher number of *Aloe* species and use-reports from the Oromo community could be attributed to the diverse ecology and the wider geographic distribution of *Aloe* species in the floristic regions inhabited by the Oromo community as compared to the other three ethnic communities. It was verified that there were no significant difference (p -value = 0.061) between the uses of *Aloe* species among the three cultural/ethnic communities (Afar, Somali, and Harari).

4.3. Ethno-medicinal and bio-cultural knowledge among gender and age categories

The practice of traditional medicine is restricted to men in Ethiopia [51, 52] and, not surprisingly, men were found to hold deeper knowledge of the medicinal uses of *Aloe*. However, women were found to be more knowledgeable on the cultural uses of aloes such as weaning children from breastfeeding, cosmetics, colouring hand and leg/tattoo, and body fumigation.

Most of the young and some adult informants were found to be less knowledgeable about the diversity of *Aloe* species and their uses as compared to majority of adults and elderly informants. This could be attributed to the current cultural transformation, and increasing accessibility of education and health centers in rural areas that has challenged the acquisition of indigenous knowledge among young people. An alternative interpretation is that knowledge accumulates over time, and that older people have had longer to learn about plant uses. Similar results were reported in some other cultural groups in Ethiopia [53, 54] and among users of *Aloe* species in Tanzania [43]. Some studies indicated that valuable ethno-medicinal information was shared with researchers mostly from informants over 60 years of age [55]. In addition, the knowledge of older people might not have been affected by the need to find new subsistence activities, and was thus preserved without external influence. For example, the dried and powdered exudate of *Aloe harlana*, *A. megalacantha* subsp. *alticola*, and *A. megalacantha* subsp. *megalacantha* locally known as *SIBRII*, which was used as colon cleaner, were sold in the open local market places of nearby towns. The processing, selling, and use of this local product is mainly restricted to elderly people. The knowledge of medicinal values of most *Aloe* species was particularly evident among elderly informants and also still retained with the majority of adults, but younger participants showed much less knowledge, and if knowledge gain has indeed slowed down, this could have negative consequences for their conservation in future.

4.4. Informant consensus factor and value indexes

The most popular indices used to evaluate the relative importance of the different species, which are used in the traditional system are based on "informant consensus," i.e., the degree of agreement among the various interviewees [40, 56]. In this respect, there was a strong consensus for the medicinal value of the leaf exudate. Efficacy of traditional medicinal plants is strongly correlated with Fic value, meaning pharmacologically effective remedies are expected to have greater Fic value, and vice versa [38]. The species with higher values in all indices of

UV, RFC, RI, and CV like *A. calidophila*, *A. megalacantha* subs. *megalacantha*, *A. gilbertii* subsp. *gilbertii*, and *A. pirottae* were identified to be the most ethno-medicinal and bio-culturally important species.

5. Conclusion

Aloe species were reported to have multiple uses, but it has been shown that the most common local use was in the traditional health care system. In priority setting for *Aloe*-based product development and cultivation, species like, *A. calidophila*, *A. megalacantha* subsp. *alticola*, and *A. pirottae* were identified to be the top three ethno-medicinal and bio-culturally important endemic *Aloe* species. In addition, unidentified *Aloe* samples could instigate taxonomic discussion and investigation. More importantly, the unique use-report: "poisonous to carnivores" from two *Aloe* species called *A. retrospiciens* and *A. ruspoliana* could initiate detailed phytochemical and toxicity studies, which have not been done so far, and are of particular importance for this study. In this study, it has been shown that the leaf exudates were highly valued in medicinal applications. Therefore, research interests on medicinal values of Ethiopian endemic aloes need to focus on exudates for phytochemical and pharmacological analysis. In addition, the result showed the unfortunate decline in bio-cultural and ethnomedicinal knowledge between generations in most of the study areas. The deterioration of indigenous knowledge coupled with declining wild populations of most *Aloe* species could stimulate an urgent ethnobotanical study for in-depth investigation before it is lost. It should be followed with phytochemical and pharmacological analyses in order to give scientific ground to the ethno-medicinal knowledge as well as to signify conservation attention and future potential utilization. In general, the output of this comprehensive ethno-medicinal and bio-cultural knowledge will encourage the community to conserve, manage, and sustainable use *Aloe* species in the natural habitat as well as through cultivation.

Declarations

Author contribution statement

A. Belayneh, S. Demissew: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

N.F. Bussa: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

D. Bisrat: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

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PAPER II

Phytochemical Isolation and Identification of Phenolic Compounds from Leaf Exudates of Ethiopian Endemic and near endemic *Aloe* Species Using HPTLC Technique

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Prepared for submission

Phytochemical Isolation and Identification of Phenolic Compounds from Leaf Exudates of Ethiopian Endemic and near endemic *Aloe* Species Using HPTLC Technique

ABSTRACT

Ethiopia, a recognized centre of diversity for *Aloe*, has 46 accepted species and three infraspecific taxa but their phytochemical profiles are least known. In view of the broad number of *Aloe* species used for herbal medicine, few have been studied for their valuable bioactive compounds. Therefore, extraction, isolation, and identification of phytochemical compounds, which would enhance knowledge base exploitation of enormous potential locked up in Ethiopian *Aloe* species. Leaf exudates from 16 endemic and near-endemic *Aloe* species were analyzed using fully automated high-performance thin layer chromatography (HPTLC). Optimizations of mobile phases were done for better separation of bands. Data were analyzed using visionCATS software and two-way ANOVA followed by univariate post hoc test for multiple comparisons. The R_f values and number of bands per track among the mobile phases showed significance difference ($P < 0.05$). The solvent system containing ethyl acetate: acetonitrile: formic acid in the volume ratio of 9:2:1v/v/v was found ideal mobile phase with 4% KOH in 96% MeOH derivatizer, which has been resulted in well-resolved bands/analytes with distinct R_f values for 14 species. However, *A. ruspoliana* and *A. retrospiciens* did not show any band. Double development of HPTLC plate has increased the visibility, optimized band separation and revealed well-resolved bands along the tracks. The most common compounds were aloin followed by microdantin and aloinoside. The phytochemical profile of *A. pirottae*, *A. megalacantha* subsp. *alticola*, and *A. trichosantha* subsp. *longiflora* were done for the first time and resulted in unique characteristic compounds. In addition, the phytochemical variation was found significantly different ($P < .05$) between the two sub species of *Aloe megalacantha*, which could raise taxonomic discussion. These findings initiated further phytochemical studies for better characterization of the isolated bands of the corresponding compounds.

Keywords: *Aloe megalacantha*, *Aloe pirottae*, aloin, aloinoside, herbal medicine, microdantin

1. INTRODUCTION

The genus *Aloe* L. had been used therapeutically and for multiple bio-cultural utility of topical and internal effects, certainly since Roman times and perhaps long before (Morton, 1961; Reynolds, 1996). Some *Aloe* species have been prominent for their medicinal and cosmetic properties that have been exploited over thousands of years (Sebsebe and Nordal, 2010; Grace *et al.*, 2009, Bjorå *et al.*, 2015). For example, in Kenya, *Aloe secundiflora*, *A. turkanensis* Christian, *A. rivaie* Baker, *A. calidophila* Reynolds, and *A. scabrifolia* L.E. Newton & Lavranos were reported to be commercially exploited for aloe bitter exudates (Lubia *et al.*, 2008; Bjorå *et al.*, 2015). In South Africa, *A. ferox* has gained popularity as an ingredient in cosmetic formulations and food supplements. In addition, the genus *Aloe* is also included in the WHO list of most used medicinal plants (WHO, 2001). In view of the broad number of *Aloe* species, 560 accepted species and 21 infraspecific taxa (The Plant List, 2013), only few *Aloe* species have been grown and commercialized at the global level. This might be due to the limited knowledge of their effective bioactive compounds, which have been reported to possess a number of phytochemical compounds of secondary metabolites from leaf and root extracts (Dagne *et al.*, 2000; Lucini *et al.*, 2015; Cardarelli *et al.*, 2017).

Most of the *Aloe* species were reported to have multiple uses but more of medicinal. Out of the total 1,467 use records for 173 accepted species and infraspecific taxa of *Aloe*, which were collated from the literatures and through personal observation, the traditional medicinal uses were described by 74% of the use records (Grace *et al.*, 2009). Numerous in vitro and in vivo pharmacological studies as well as clinical trials have been also confirmed the traditional uses of *Aloe* including wound healing, anti-inflammatory, anti-microbial, anti-plasmodial, anti-malarial, anti-oxidant, anti-ulcer, anti-diabetic or hypoglycemic, anti-cancer, anti-viral, and anti-hyperlipidemic activities (Chen *et al.*, 2012; Grace *et al.*, 2008; Radha and Laxmipriya, 2015; Gelila *et al.*, 2011; Anwar *et al.*, 2014; Biruktawit *et al.*, 2015; Tibebu *et al.*, 2018). It has been reported that the medicinal applications were ascribed more from exudates of *Aloe* leaves (Lucini *et al.*, 2015; Cardarelli *et al.*, 2017). The popularity of exudate uses for diverse medicinal formulations to treat different health problems could be related with the higher number of different phytochemical constituents in exudates of *Aloe* species. That is why most of the

research on *Aloe* species were focused on *Aloe* leaf exudates related to its medicinal uses (Gelila *et al.*, 2011; Anwar *et al.*, 2014; Biruktawit *et al.*, 2015; Tibebu *et al.*, 2018).

In Ethiopia, 46 species with three subspecies were identified and documented, among which 35 were endemic and near endemic (i.e. have restricted distribution in one or few neighboring countries) to the country (Edwards, *et al.*, 1997; Sebsebe *et al.*, 2001; Sebsebe and Nordal, 2010). There are anticipated several interesting aspects of the Ethiopian *Aloe* species that need further studies, and the endemic aloes represent an economic potential (Sebsebe and Nordal, 2010). Some research works done in Ethiopia also indicate that the local communities make use of them for traditional medicines to treat major health problems like, *A. pirottae* for malaria (Anteneh *et al.*, 2012), *A. harlana* for tropical ulcer (Anteneh and Negussie, 2014), *A. sinana* as wound-healing agent, insecticide, treatment of snake-bite and malaria (Genet *et al.*, 2014). Following such indigenous knowledge related to the bio-cultural uses, few of the endemic *Aloe* species (like, *A. harlana*, *A. trichosantha*, *A. gilbertii*, and *A. citrina*) were investigated for their bioactive phytochemical compounds and antioxidant properties. Such isolation and characterization, and efficacy evaluation of bioactive phytochemical compounds were validated the existing traditional uses of aloes as well as the custodian of new compounds (Dagne *et al.*, 2000; Gelila *et al.*, 2011; Anwar *et al.*, 2014; Yitagesu *et al.*, 2014; Biruktawit *et al.*, 2015; Mekdes *et al.*, 2015).

That is why, extensive trials are necessary to demonstrate the effectiveness of a bioactive compound to verify the claimed traditional values of aloes in Ethiopia. The aim of this chapter was to analyze the phytochemistry of targeted *Aloe* species using high performance thin layer chromatography (HPTLC). HPTLC is a sophisticated and automated form of thin-layer chromatography (TLC) with precise sample application, better and advanced superior separation efficiency using optimized coating material, detection limits and the possibility of analyzing several samples simultaneously on the same plate (Sheikh *et al.*, 2013; Pandey *et al.*, 2016). Therefore, using advanced techniques of HPTLC, isolation and identification of compounds from the leaf exudates of targeted *Aloe* species have been analyzed to foster further phytochemical characterization of the identified compounds from Ethiopian *Aloe* species.

2. MATERIALS AND METHODS

2.1. Plant Collection and Authentication

Fresh leaves of *Aloe* species from east and south of the rift valley floristic regions of Ethiopia were collected in their corresponding natural habitats. Their taxonomic identification and authentication were done in the National Herbarium of College of Natural Sciences, Addis Ababa University (AAU). Voucher specimens were deposited at AAU and Haramaya University (HU) Herbaria as well as live plants were kept in the garden of HU compound.

The fresh leaves of each *Aloe* species were used for exudate extraction. Exudates from the fresh leaves of targeted *Aloe* species were collected by cutting the leaves transversally near the base and arranging them concentrically around plastic plates to drain exudate for 10-20 minutes. Then exudates of each species were poured in airtight plastic bottles, kept in field ice bag and brought to Lab for storage in the refrigerator at 4° C until proceed to freeze-drying. The extracts were protected from any possible oxidation by being stored in airtight aluminium foil covered flasks. Then each exudate were freeze dried (Christ Alpha 1-2 LDplus freeze drier, Germany) to be crystallized and powdered, labelled and stored at 4° C until used for phytochemical analysis and toxicity test.

2.2. Chemicals

The major chemicals used are like, Acetonitrile (CH_3CN), chloroform/trichloromethane (CHCl_3), ethyl acetate/EtOAc ($\text{C}_4\text{H}_8\text{O}_2$), methanol/MeOH (CH_3OH), formic acid (CH_2O_2), potassium hydroxide (4% KOH), 2-aminoethyl diphenyl borinate (1.5gm) in 150 ml MeOH, and polyethylen glycol 6000 (7.5 gm) in 150 ml ethanol for natural product derivatizer preparation and ultrapure water purified with a NaNo pure Diamond UV system (Barnstead International, Dubuque, IA, USA). All chemicals used for mobile phase were HPTLC analytical grade from Burdick & Jackson (Muskegon, MI, USA) and from Sigma-Aldrich (St. Louis, MO, USA).

Standards used in optimization of HPTLC parameters were also standard grades from Tshwane University of Technology (TUT), Department of Pharmaceutical Sciences laboratories where this experiment were done in Pretoria, South Africa. Some available standards were used like,

aloin A and B, aloinoside A and B, microdontin A and B, p-coumaric acid, aloeresin A, aloeresin D, and plicataloside from TUT, which have been isolated from *Aloe ferox*, *A. dolomitica*, *A. marlottii*, and *A. thraskii* (Provided by Prof. Alvaro Viljoen). The purity of these reference compounds were standard grades of higher than 95%.

2.3. HPTLC Equipments

The HPTLC system was all composed of CAMAG supplies such as, automatic TLC sampler 4 (CamagMuttentz, Switzerland) with the syringe, 100 μ L (Hamilton Bonaduz, Switzerland) spotting device (ATS 4); the densitometer consisted of CAMAG TLC scanner-3 provided with visionCATS HPTLC software (version: 1.4.4.6337), automatic developing glass twin trough chamber (20 x10 cm); automated derivatizer, TLC plate heater II, and TLC visualizer 2 (Figure 1). The stationary phase was composed of pre-coated silica gel 60 F₂₅₄ HPTLC plates (20 cm x 10 cm; with 0.25 mm thickness) (E. Merck) with aluminum sheet support.

2.4. Sample Preparation

A 30 mg powder exudate sample in 3 mL methanol (MeOH) was dissolved for each sample to prepare the test solution and sonicated (40 kHz, 105 W) at room temperature using an ultrasound machine (Mujigae, Seoul, Korea). The resultant dissolved solution was transferred to 2 ml vial for CAMAG HPTLC auto-sampler. The rest 1 ml sample was used for repeated manual TLC for mobile phase optimization using TLC plate in development chamber.

2.5. HPTLC Procedure

HPTLC plates were cleaned by predevelopment with methanol in ascending method, before spotting. Then the vials containing samples and HPTLC plate of 20 cm $\times$ 10 cm HPTLC silica gel 60 F₂₅₄ was immersed in a CAMAG Automatic TLC Sampler IV glass chamber (20 cm $\times$ 10 cm), containing 30 mL methanol (HPTLC grade) as solvent system for automated spotting. The chamber was covered with glass lid and left until development of the plate to the top with methanol. Samples were administered to the HPTLC plates as 8 mm band width, 3 mm between bands, and 8 mm from the bottom of the plate via Linomat-5 automatic sample applicator (with

nitrogen flow) equipped with a 100 μ L Hamilton syringe. Volumes applied was 2.0 μ L with the syringe rinsed with absolute methanol between samples. Delivery rate from the Hamilton syringe was fixed at 100 nl/s (Loescher *et al.*, 2014; Pandey *et al.*, 2016).

After complete spotting, the plate was removed from TLC glass chamber and dried in oven at 105⁰ C for 5 minutes then was put in developer, which consistent of the mobile phase; ethyl acetate (EtOAc): Acetonitrile (CH₃CN): formic acid (CH₂O₂) (9:2:1 v/v/v) selected after several optimization trial. The developing chamber was adjusted at room temperature (25⁰ C) and 50% relative humidity in a CAMAG twin trough glass chamber (20 cm $\times$ 10 cm) saturated earlier with mobile phase vapour for 20 minutes and the plate was preconditioned for 5 min before the development. Once development was completed (Chromatograms were developed for 9.0 cm) in Camag Automatic Developing Chamber (ADC-2), a photo was taken with a 'TLC visualizer 2' containing a 12 bit camera (CAMAG) under white light and UV wavelengths of 254 nm and 366 nm before derivatization. The plate was derivatized by spraying 4% potassium hydroxide in methanol (96% MeOH) using Camag Derivatizer and then bands/spots were detected by heating the plate on Camag TLC plate heater at 100 $^{\circ}$ C after derevatization for 5 minutes. Finally, densitometric screening was performed by using Camag TLC Scanner IV at 366 nm and VisionCATS software in fluorescence mode after derivatization. The slit dimensions were 5 mm $\times$ 0.45 mm and the scanning speed was 100 nm/s. The results were observed and captures under white light and 366 nm under Camag TLC visualizer 2 (Loescher *et al.*, 2014; Ojha and Kumar, 2012; Pandey *et al.*, 2016).

2.7. Data Analysis

VisionCATS software fixed with Camag TLC Scanner IV was used for HPTLC data analysis. Two-way ANOVA followed by univariate post hoc test for multiple comparisons of means using the R package multcomp (<https://CRAN.R-project.org/package=carData>) and Tukey's Honest Significant Differences (HSD). P-value of less than 0.05 was taken as indicative of a statistically significant difference.

3. RESULTS

3.1. Optimization of the HPTLC Mobile Phase

The mobile phases are determinant factors for accurate spotting of separated bands (analyte compounds) in all chromatographic techniques. In this study, different mobile phases and derivitizers were tested for better separation and accuracy of the bands. The two most common solvent systems (ethyl acetate: methanol: water and ethyl acetate: acetonitrile: formic acid) often used in the phytochemical analysis of *Aloe* exudates as mobile phase were tried with different ratios in order to optimize the accuracy of bands' separation (Table 1).

Table 1. A number of optimization trials with two solvent systems (ethyl acetate: methanol: water and ethyl acetate: acetonitrile: formic acid) of different ratios.

Solvent system & derivatizer	Ratio v/v/v	Bands	Peak final length moved
Ethyl acetate: methanol: water & natural product	100:16.5:13.5 (23:4:3)	Compressed and concentrated at the lower part	Moved half way only
	80: 20: 10 (8:2:1)	Overlapped at the middle	Moved 5.5 cm
	80: 10: 10 (8:1:1)	*Few unresolved & concentrated at lower part	Moved 5.5 cm
	72: 12: 9	Some unresolved and concentrated at the upper part	Moved 6.8 cm
	70: 17: 13	All moved at the top and concentrated at upper part	Moved 7.5 cm
	69: 12: 9 (23:4:3)	*Resolved with some overlap	Moved 7.2 cm
	66: 15: 9 (22:5:3)	Few unresolved and most are concentrated at the middle	Moved 6.0 cm
	63: 22.5: 4.5 (7:2.5:0.5)	Most are unresolved	Moved 6.5 cm
	63: 18: 9 (21:6:3)	All concentrated at the upper limit	Moved 6.2 cm
Ethyl acetate: acetonitrile: formic acid & 4% KOH in 96% MeOH	60: 20:10 (6:2:1)	Some overlap and moved about half way along the track	Moved 5.5 cm
	60: 25: 5 (6:2.5:0.5)	Some overlap and all are concentrated at the lower part	Moved 4.6 cm
	70:20:10 (7:2:1)	Much overlap/unresolved	Moved 7.6 cm
	80:20:10 (8:2:1)	Well resolved with few overlap	Moved 8.2 cm
	90: 20: 10 (9:2:1)	**Well resolved	Moved 8.5 cm

*Better resolved among all the trials under ethyl acetate: methanol: water; **best resolved under ethyl acetate: acetonitrile: formic acid mobile phase

The spots appeared more compact and peak shape more symmetrical when the TLC plate were pre-treated first with ethylacetate: methanol: water (100:16.5:13.5 v/v/v) and sprayed with natural product derivatizer. Of course, this ratio of ethyl acetate: methanol: water (100:16.5:13.5) was well revealed the bands up to R_f (retention factor) value of 0.5 but could not detected any of the compounds above R_f 0.5 (Figure 1). In this solvent system the ratios 80: 10: 10 (8:1:1 v/v/v) and 69: 12: 9 (23:4:3 v/v/v) were found better-resolved bands but still few overlapped at the middle. HPTLC profile through EtOAc: acetonitrile: formic acid mobile phase with 4% KOH in MeOH derivatizer under the ratios of 60:20:10 and 60: 25: 5 resulted in wider unresolved yellow bands at R_f ±0.4, unresolved yellow bands at R_f 0.61, and did not detected components above R_f 0.75 values (Figure 1). Among the various ratios tried for the two solvent systems as a mobile phase, the ethyl acetate: acetonitrile: formic acid in the volume ratio of 9:2:1 v/v/v with 4% KOH in 96% MeOH derivatizer resulted in well-resolved and much better bands with distinct R_f values (Figure 1). To the knowledge of the authors, this mobile phase has not been used so far for the phytochemical analysis of Ethiopian *Aloe* species with HPTLC system.

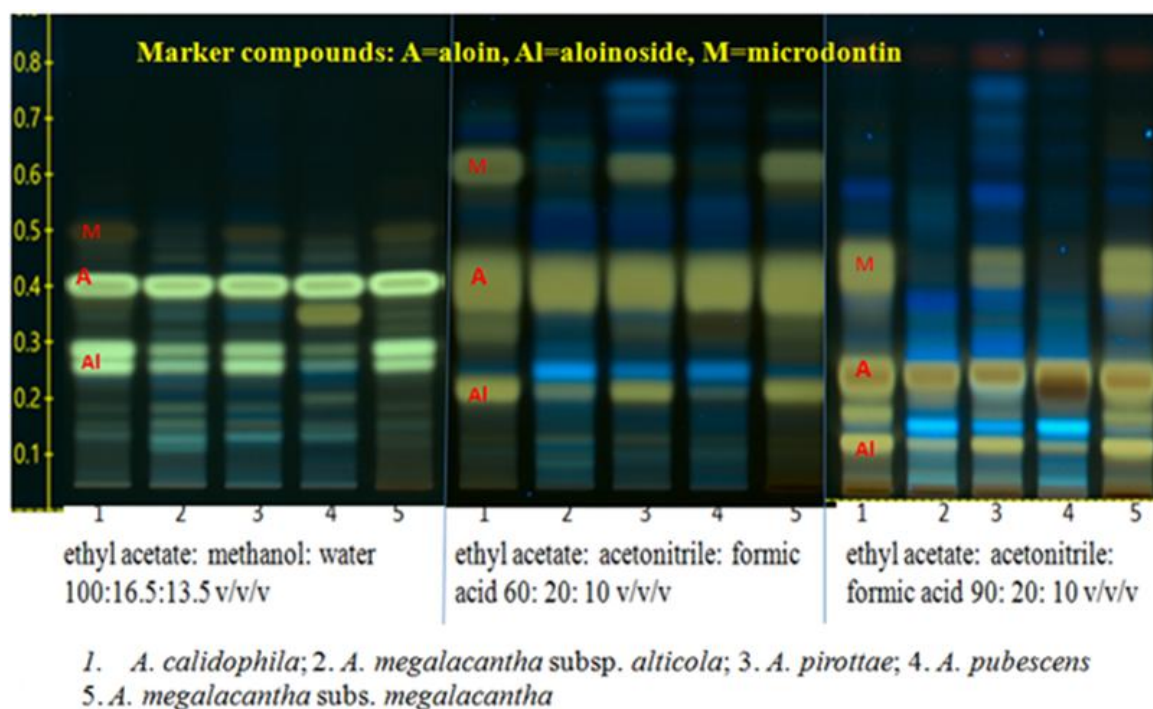


Figure 1. Comparison of the mobile phase optimization trial with three different ratios of the two solvent systems, in which the last five tracks with ethyl acetate: acetonitrile: formic acid (90:20:10 v/v/v) resulted in well-resolved bands.

In order to assess the extent of variation with different ratios of same mobile phase, plates were set up and run under same automated instrument/condition. Then a two way ANOVA with repetition (n=3) was found that the results (Rf values and band numbers per track) were statistically different (p=0.03).

3.2. HPTLC Detection and Identification of Compounds

3.2.1. Detection of Compounds

High performance thin layer chromatography (HPTLC) fingerprinting of the leaf exudates from 16 endemic and near endemic *Aloe* species were run on silica gel plates and developed in a mobile phase of ethyl acetate: acetonitrile: formic acid (90:20:10 v/v/v) with 4% potassium hydroxide (KOH) in 96% methanol (MeOH) derivatizing agent to maximize the visibility chemical fingerprints. The samples were run with some standards of pure compounds (e.g. Aloeresin A) and by comparison with external standards of previously isolated from South African *Aloe* species (*Aloe ferox*, *A. marlottii*, *A. arborescense*, *A. thraskii*, *A. rupestris*, and *A. dolomitica*) using similar methodological conditions. The plates were viewed under white light, UV 254 nm, and UV 366 nm both before and after derivatization.

The HPTLC chromatogram has been produced a number of well-resolved bands with different retention factor (Rf). The number of bands and their visibility under white light is much lower before derivatization (Figure 2A) than after derivatization (Figure 2B). Under derivatization, the yellow-orange, brownish-orange, and pinkish bands were increase the visibility and widespread across the tracks except tracks 10, 12, and 15 (standard aloeresin A) (Figure 2B). In all HPTLC chromatograms of this chapter, the y-axis is the Rf value and the x-axis is 14 *Aloe* species and a sample standard: 1. *A. calidophila*; 2. *A. megalacantha* subsp. *alticola*; 3. *A. pirottae*; 4. *A. pubescens* 5. *A. megalacantha* subs. *megalacantha*, 6. *A. yavellana* 7. *A. harlana* 8. *A. secundiflora* 9. *A. trichosantha* subsp. *longiflora* 10. *A. otallensis* 11. *A. rivae* 12. *A. rugosifolia* 13. *A. tewoldei* 14. *A. mcloughlinii* 15. standard Aloeresin A. However two species, *A. ruspoliana* and *A. retrospiciens* did not showed any bands, which are not included on the chromatogram figures.

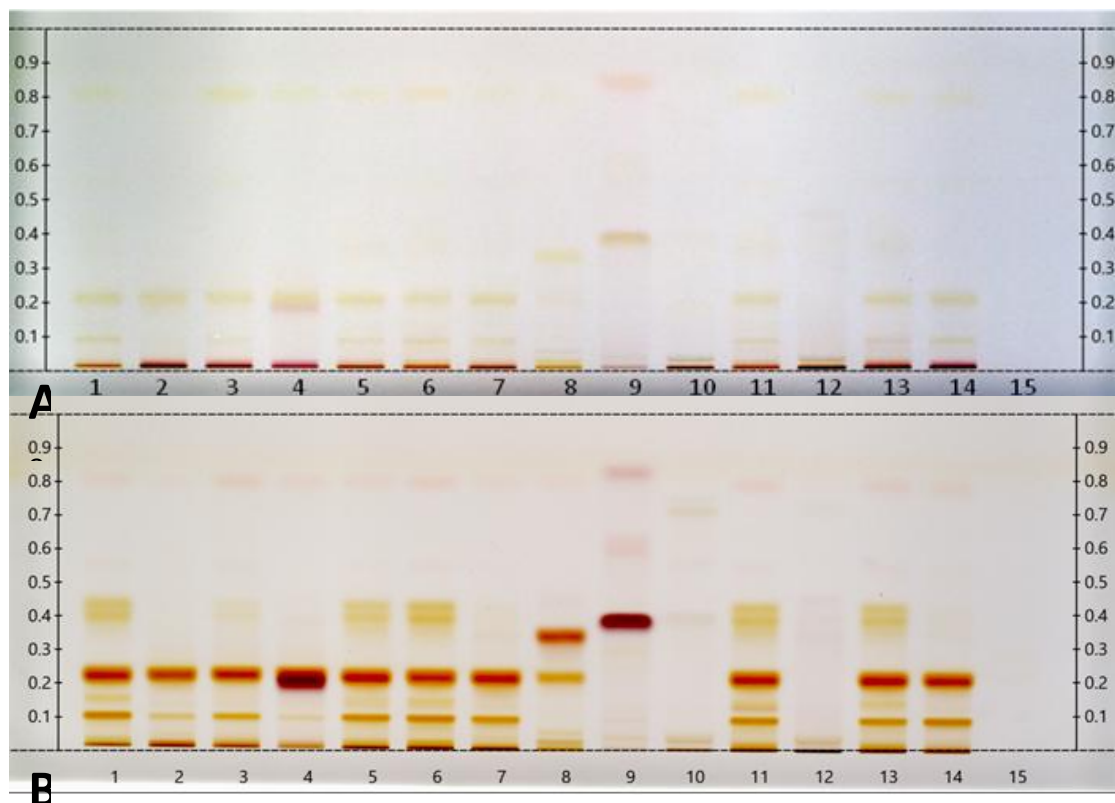


Figure 2. A developed HPTLC plate of leaf exudates from 14 *Aloe* species (track 1-14) and standard (track 15 = Aloeresin A) under white light before derivatization (A) and after derivatization (B).

The pre-derivatized HPTLC chromatogram observed under UV 254 nm appeared with more number of blackish and blackish-ash bands in most of the tracks (Figure 3). Few additional bands were detected under this UV spectrum in addition to those observed under the white light.

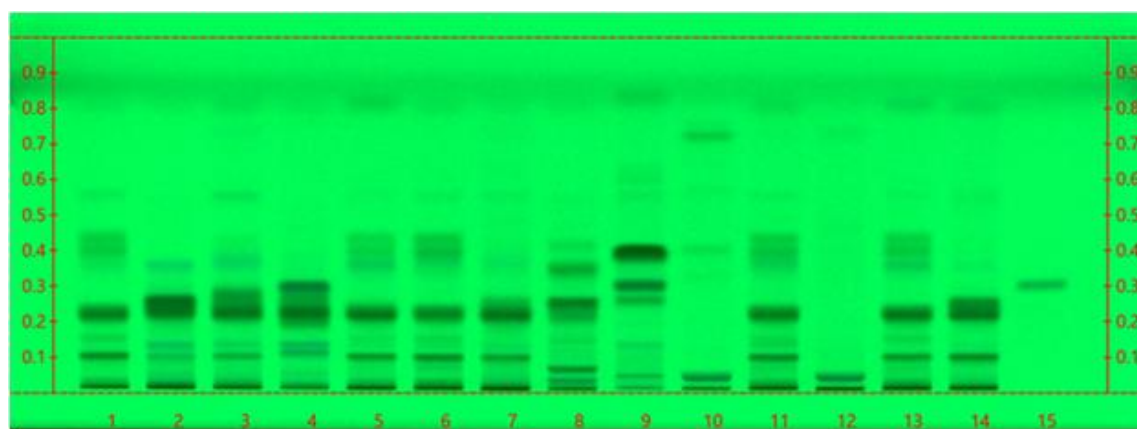


Figure 3. HPTLC chromatograms of leaf exudates from 14 *Aloe* species (track 1-14) and standard (track 15 = Aloeresin A), under UV 254 nm before derivatization.

The number and visibility of bands have been much increased under UV 366 nm after derivatization (Figure 4B) compared to pre-derivatization (Figure 4A). The HPTLC chromatogram observed under UV 366 nm, appeared with diversified coloured bands dominated by yellow and blue bands indicating more number of compounds in most of the *Aloe* leaf exudates (Figure 4 A and B). The maximum number of bands were about 14 for track 3 (*A. pirottae*) and the minimum were two for track 12 (*A. rugosifolia*) (Figure 4B).

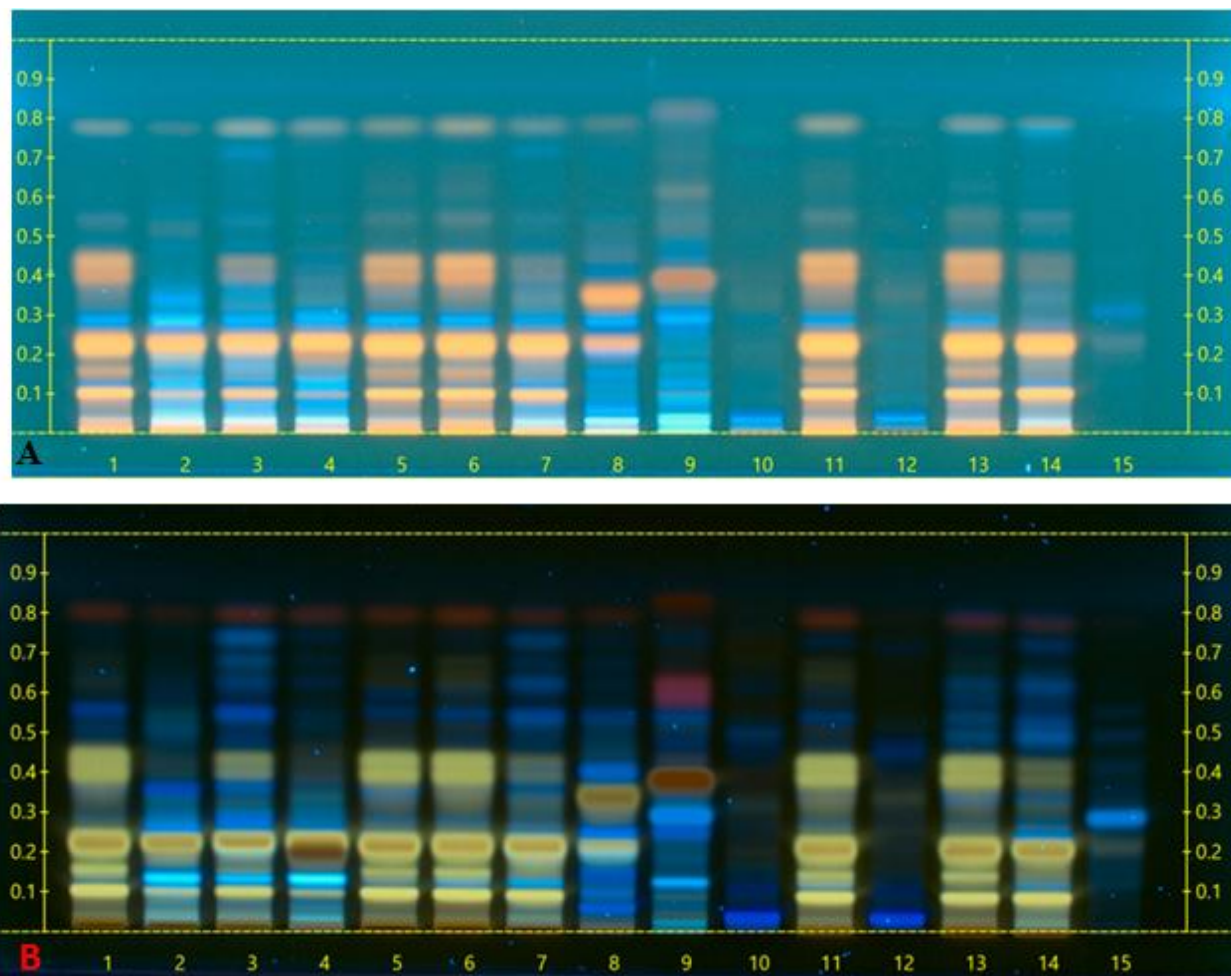


Figure 4 A and B. A developed HPTLC chromatograms for 14 *Aloe* species and a standard Aloeresin A under UV 366 nm before derivatization (A) after derivatization (B) (y-axis is the Rf value and x-axis is *Aloe* species: 1. *A. calidophila*; 2. *A. megalacantha* subsp. *alticola*; 3. *A. pirottae*; 4. *A. pubescens* 5. *A. megalacantha* subs. *megalacantha* 6. *A. yavellana* 7. *A. harlana* 8. *A. secundiflora* 9. *A. trichosantha* subsp. *longiflora* 10. *A. otallensis* 11. *A. rivae* 12. *A. rugosifolia* 13. *A. tewoldei* 14. *A. mcloughlinii* 15. standard Aloeresin A).

3.2.2. Identification of HPTLC Detected Compounds

The identification of compounds from HPTLC chromatogram was done based on the respective Rf values, standards, compared with pre-characterized South African *Aloe* species in the same condition, and colors of bands. Like most *Aloe* species, the HPTLC bands were dominated with anthrone and chromones. In addition, few anthraquinones, coumarins, pyrans and pyrones, and benzene, naphthaline and furan derivatives were also isolated from the 14 tracks (Table 2). The most common anthrones like, aloin A and B, aloinoside A and B, and microdontin A and B as well as aloeresins (chromones) were determined based on standards and comparison with pre-characterized South African *Aloe* species (in same conditions) used in this study.

Table 2. Interpretation of each track/*Aloe* species with Rf value, colour, and the corresponding compound type

Rf value	Band color	Compound type	Letter on track	Track number/ <i>Aloe</i> species
0.03	Brilliant blue	Plicataloside	P	10 & 12
0.11	Canary-yellow	Aloinoside A and B	Al	1,2,3,4,5,6,7,11, 13 & 14
0.13	Sky blue	Aloesin	D	2,3,4, 9
0.19	Chicory	Aloe-emodin-8-O-glucoside	E	4
0.22	Mellow yellow	Aloin A	A	1,2,3,4,5,6,7,8, 11, 13, 14
0.23	Mellow yellow	Aloin B	A	1,2,3,4,5,6,7,8, 11, 13, 14
0.28	Blue	Aloeresin D	R	2,3,7, 8,14
0.29	Blue	Aloeresin A	C	9
0.34	Chicory	Isoaloeresin D	X	8
0.39	Ash brown	6'-O-coumaroylaloetin	U	9
0.39	Canary yellow	Microdontin A	M	1,3,5,6,7,11,13,14
0.43	Canary yellow	Microdontin B	M	1,3,5,6,7,11,13,14
0.40	Blue	Aloenin B	N	8
0.55	Light blue	p-Coumaric acid derivatives	Pc	1,3,6,7,8,9,11,13,14
0.61	Light pink	Homonataloside A/B	H	9
0.81	Pinkish	Aloe-emodin	B	1,2,3,4,5,6,7,8,11,13,14
0.83	Light pink	7-O-Methylaloeresin A	Mt	9

The most common compounds were aloin A/B and aloe-emodin from 11 species, aloinoside A/B from 10 species and microdontin A/B from eight species, which are all anthrones and anthraquinones. The common chromones were aloeresin A and D isolated from six *Aloe* species, and p-coumaric acid derivatives (coumarin) from nine species (Figure 5).

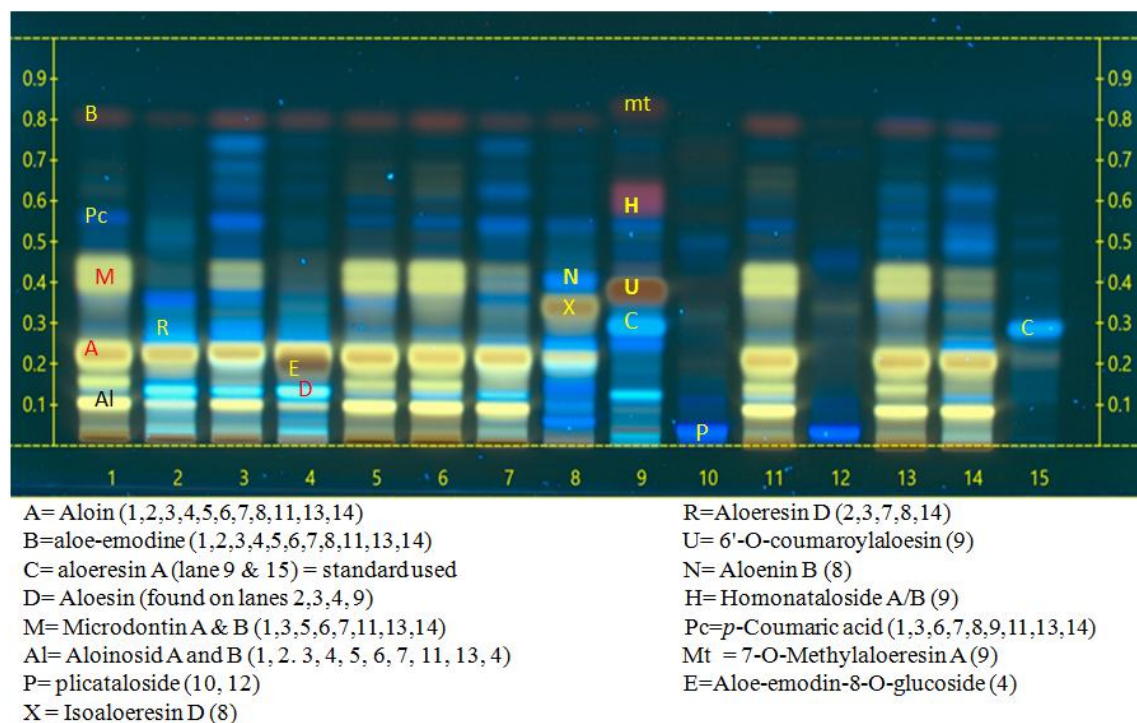


Figure 5. Some of the bands characterized as compounds on the HPTLC chromatogram

3.3. Double Development of HPTLC Plates

This is an innovative method of double development of HPTLC plate in automatic developing chamber followed with derivatization. Despite the fact that the R_f values increased due to double development, this method has been well separated few of the overlapped bands like, $R_f = 0.41-0.43$. In addition, a brilliant pink band overlapped with $R_f = 0.41$ on track 4 (*A. pubescens*) under white light and ash-brownish band under UV 366 nm was well-resolved with this innovative method of double development (Figure 6A & B).

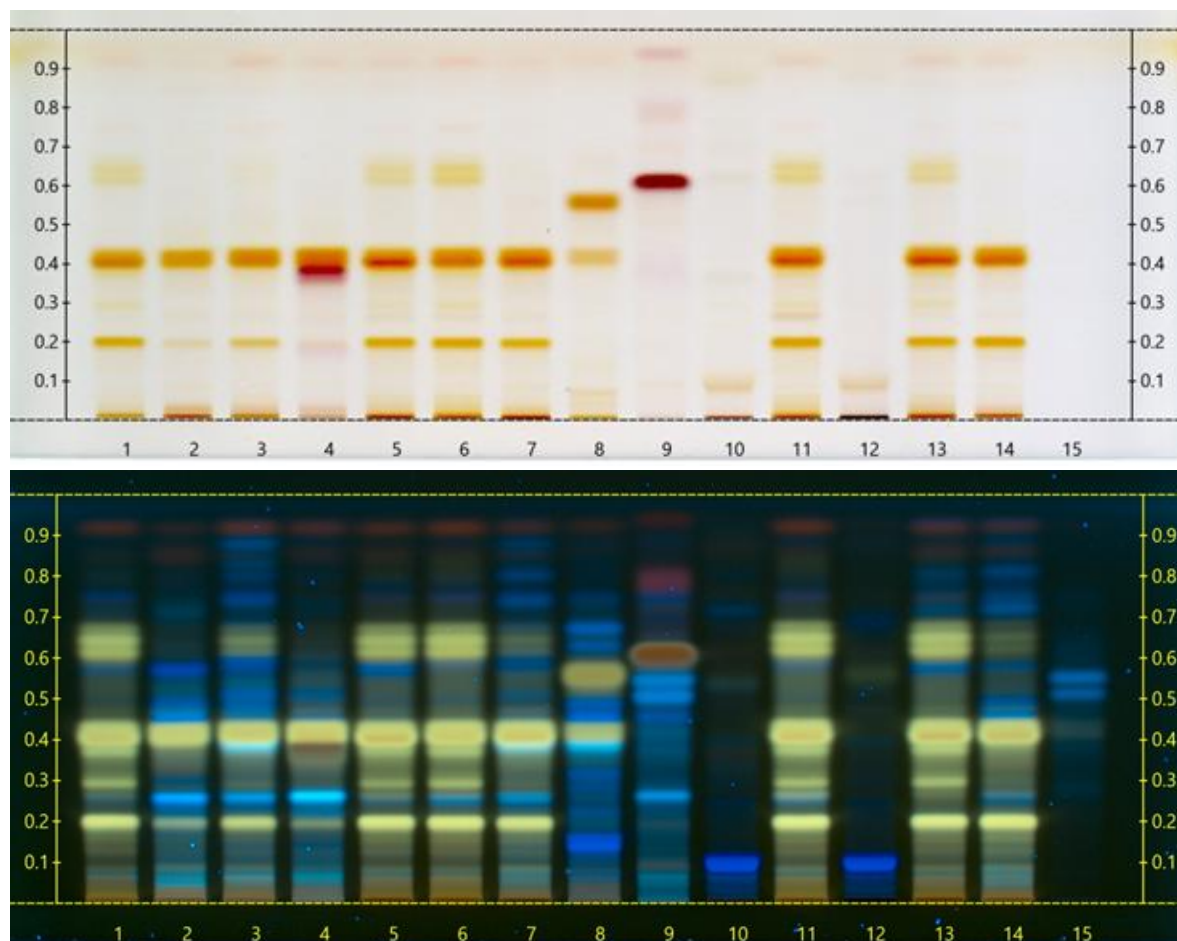


Figure 6. A developed HPTLC plate of 14 *Aloe* species and a standard Aloeresin A, which was developed twice (double development) followed with derivatization and viewed under white light (A) UV 366 nm (B) resulted in well resolved bands

4. DISCUSSION

4.1. Optimization of the HPTLC Mobile Phase

It is well known that the solvent system does exert a profound influence on the quality and shape/symmetry of the HPTLC bands. Selection of mobile phase is trial and error processes supported with literature reports opt to optimize the reported mobile phases (Viljoen *et al.*, 1999; Chen *et al.*, 2009). One's own experience and literature reports could support to develop better mobile phases with respect to specific sample types. There are a number of reports used different mobile phases and ratios in phytochemical analysis of *Aloe* exudate like, ethyl acetate: methanol: water (100:16,5:13,5 v/v/v) in HPLC (Pandey *et al.*, 2016); ethyl acetate: methanol: water in the ratio of 77:13:10 v/v/v (Dagne *et al.*, 2000); ethyl acetate: methanol: water in volume ratio of 100:13.5:10 v/v/v with a natural product derivatizer (Rebecca *et al.*, 2003). In this study, the mobile phase ethyl acetate: acetonitrile: formic acid in the volume ratio of 9:2:1 v/v/v with 4% KOH in MeOH derivatizer found more efficient in phytochemistry analyses of *Aloe* leaf exudates compounds with HPTLC technique. All *Aloe* leaf exudate samples constituents (analyte compounds) were clearly resolved without any tailing and diffuseness. Therefore, in HPTLC studies for Ethiopian *Aloe* species, this mobile phase was found ideal with 4% potassium hydroxide (KOH) in 96% methanol (MeOH) derivitizer.

4.2. HPTLC Isolation and Identification of Phytochemicals

In this study, the HPTLC fingerprinting revealed remarkable variation in the chromatographic profiles of the targeted *Aloe* species. Much variation in number and visibility of bands were observed under white light before and after derivatization. The less visible yellow-orange ($R_f = 0.22$) and pinkish ($R_f = 0.81$) bands before derivatization were well-resolved after derivatization under white light. Similarly, the less visible bands at R_f 0.34 on track 8, R_f 0.39 and 0.85 on track 9, and R_f 0.1 in most of the tracks were also well detected under white light after derivatization. It was clear that spraying with 4% potassium hydroxide (KOH) in 96% methanol (MeOH) derivatizing agent has been maximized the visibility of chromatogram (chemical fingerprint). In both conditions, track 10 (*A. otallensis*) and track 12 (*A. rugosifolia*) did not showed any spots under white light indicating that the limited compounds in these two *Aloe*

species were not detectable under white light. Derivatization could achieve better detection and visibility of the spots along the lanes on HPTLC plate (Loescher *et al.*, 2014).

The brownish band with Rf 0.34 on track 8 (*A. secundiflora*) and the deep pink band with Rf 0.39 on track 9 (*A. trichosantha* subsp. *longiflora*) are much visible after derivatization and looks unique compounds for the corresponding species of the tracks. The blue band at Rf 0.29, ash-brown band with Rf value 0.39, light pink double bands at Rf 0.59 and 0.62, and light pink band on the top at Rf 0.83 situated on tracks 9 (*A. trichosantha* subsp. *longiflora*) are all unique only for this species that makes it much different from the targeted *Aloe* species in this study. Track 8 known as *A. secundiflora* also showed some unique colored bands with different Rf values like, 0.04 and 0.34, which are not shared with any one of the tracks (*Aloe* species). Some of these unique compounds could have taxonomic relevance. For example, A6'-O-coumaroylaloetin [2-acetyl-8-(6-O-coumaroyl-beta-D-glucopyranosyl)-7-hydroxy-5-methylchromone], a mono-ester chromone derivative was reported as a taxonomic marker for *Aloe* section *Anguialoe*, which has been isolated from *Aloe castanea* (Heerden *et al.*, 2000). In general, the HPTLC chromatogram has been produced more number of well-resolved bands with different retention factor (Rf) under UV 254 nm. Those invisible bands of track 10 (*A. otallensis*), track 12 (*A. rugosifolia*), and track 15 (standard Aloeresin A) under white light were observed under UV 254 nm. Therefore, the numbers of visible bands per track were increased under this spectrum.

The numbers of clearly isolated compounds from the HPTLC chromatogram of 14 *Aloe* species were higher. However, partial numbers of bands were identified using the available standards and literature sources. The common anthrones like aloin A and B, aloinoside A and B, aloemodin, and microdantin A and B were identified from most of the tracks with their respective Rf values. The yellow band at Rf 0.22-0.23 are aloin A and B, pinkish band at Rf 0.81 is aloemodin, the double yellow bands at Rf 0.39-0.43 are aloinoside A and B. These compounds are the major constituents of commercial *Aloe* species like *A. ferox* and *A. barbadensis* (Pandey *et al.*, 2016; Sánchez-Machado *et al.*, 2017; Salehi *et al.*, 2018). It was revealed that most of the *Aloe* leaf exudate (tracks 1-8, 11, 13, and 14) contain aloin A and B with Rf 0.22, which appeared as mellow-yellow under UV 366 after derivatization. It was visually evident from the

intensity that these bands (Rf 0.22 on tracks 1-8 and 11, 13, and 14) have relatively higher concentration of aloin, which has been confirmed with UHPLC–MS analysis. The double bands (canary yellow) at Rf 0.039 - 0.41 characterized as microdontin A and B are also detected on track 7 and 14 after derivatization in addition to those tracks under white light (tracks 1, 3, 5, 6, 11, and 13). Whereas, aloin band was not detected from tracks 9 (*A. trichosantha* subsp. *longiflora*), track 10 (*A. otallensis*) and track 12 (*A. rugosifolia*). This could be due to the absence or the very minimum concentration of aloin in these species.

The brilliant blue band at Rf value 0.03 on tracks 10 (*A. otallensis*) and 12 (*A. rugosifolia*) was identified as plicataloside that make these species different from the rest the *Aloe* species. As stated by Viljoen (1999) plicataloside is a single marker compound having chemotaxonomic values indicating the taxonomic relationship between 20 tropical east African *Aloe* species. plicataloside was the only bioactive compound identified from *A. otallensis* and *A. rugosifolia* (Viljoen *et al.*, 1999; Biniam *et al.*, 2011). However, in this study these two *Aloe* species showed additional colored bands, which were not be able to be identified from previous reports of HPLC chromatogram. In addition, the chromones like aloeresins and aloesins as well as the pyrans and pyrons like aloenins were identified. The blue band on track 9 at Rf 0.29 is aloeresin A (like standard on track 15), sky blue band at Rf 0.13 on bands 2, 3, 4, and 9 is aloesin. The blue band at Rf 0.4 on track 8 was identified as aloenin, which is found only on this track. The HPTLC fingerprint of the two subspecies of *Aloe megalacantha* i.e. *Aloe megalacantha* subsp. *megalacantha* and *A. megalacantha* subsp. *alticola* revealed significance difference in their phytochemical profiles. The higher HPTLC chromatogram variation observed between these two subspecies of *Aloe megalacantha* could attract further taxonomic discussion.

There are a number of HPTLC bands, which are not characterized in this study due to lack of enough standards. Characteristic HPTLC fingerprinting of particular plant species will not only help in the identification and characterization of a particular species with its phytochemical profile but could provide basic information useful for the isolation, purification, and characterization phytochemical compounds of the plant species (Shuayprom *et al.*, 2014). Therefore, further isolation, characterization and quantification of compounds were done using UHPLC-MS and DAD UV spectrum analysis.

3.3. Double Development of HPTLC Plates

The double development carried out in automatic developer chamber followed with derivatization and scanned under UV 366 has been increased the visibility, optimized the band separation, and revealed well-resolved bands along the tracks. Interestingly, almost all the bands including few of the overlapped bands have been well-resolved after double (twice) development followed with derivatization. Therefore, where there is an overlap of bands/compounds the double development could be an option for further separation of the bands in *Aloe* phytochemical analysis. HPTLC plates with double development using two solvent systems resulted in optimum separation of the spots from leaf extracts of *Capparis spinosa* (Vaidy *et al.*, 2018).

5. CONCLUSION

In HPTLC studies, the solvent system does exert a profound influence on the quality and shape of the HPTLC bands. So that, ethyl acetate: acetonitrile: formic acid in the volume ratio of 9:2:1 v/v/v was found ideal as mobile phase with 4% potassium hydroxide (KOH) in 96% methanol (MeOH) derivatizer for Ethiopian *Aloe* species in HPTLC studies. Because this mobile phase has been increased the visibility, optimized the band separation, and revealed well-resolved bands along all the tracks. More importantly, the HPTLC fingerprinting has been revealed remarkable variation in the chromatographic profile of the targeted *Aloe* species exudates. The wellknown compounds common to most *Aloe* species like aloin A and B, aloinoside A and B, and microdantin A and B were determined with clearly observable bands of different colours. Those *Aloe* species with higher number of medicinal values were found to possess more number of bands corresponds to compounds, in which the synergetic effects could have been maximized the traditional medicinal values. The bands were more visible and increased in number after derivatization and UV spectrum of 366 nm. The different colour bands have been indicated the different compounds. This could lead to further characterization of each bands/compounds using UHPLC-MS-DAD technique. Moreover, it was visually evident from the intensity of the bands that some tracks have higher concentrations of compounds like aloin (mellow yellow) and microdantin (canary yellow). Even though most *Aloe* species share some common compounds, more of anthrones, between and among themselves, indicating some degree of relationships in their chemical composition, much variation were also observed in the distribution of typical aloin compounds in leaf exudates. In this study, unique types of compounds were isolated, which were not reported so far from the Ethiopian *Aloe* species. More interestingly some *Aloe* species have compounds unique to them and not shared with any of the *Aloe* species targeted in this study. These could attract more research interest for their biological activities. In addition, lack of detectable constituents from both *Aloe retrospiciens* and *Aloe ruspoliana* in this study could attract further investigation for their phytochemical characterization and may be taxonomic discussion. The more visible bands, higher number and unique compounds isolated from *Aloe* species targeted in this study foster further phytochemical techniques for characterization and quantification of each isolated compounds from HPTLC technique.

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PAPER III

Phytochemical Characterization of Leaf Exudates of Ethiopian Endemic *Aloe* Species Using UHPLC-MS-DAD Techniques

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Phytochemical Characterization of Leaf Exudates of Ethiopian Endemic *Aloe* Species Using UHPLC-MS-DAD Techniques

ABSTRACT

Over the years, interest in the phytochemical profile of *Aloe* species has been on the rise, due to its popularity as an ingredient in pharmaceuticals, cosmetic formulations, and food supplements. Limited number of *Aloe* species have been grown and commercialized at the global level, but none has been from the 46 Ethiopian *Aloe* species; that might be due to the limited knowledge of their valuable compounds. Therefore, isolation, characterization, and quantification of phytochemical compounds were done for targeted *Aloe* species. Leaf exudates from 16 *Aloe* species were analyzed using ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) coupled with ultraviolet-photodiode array detection (UV-DAD) equipped with electrospray ionization (ESI) (Waters® Acquity). Data were analyzed using Masslynx (version 4.2) software. The UHPLC-MS chromatogram of 16 *Aloe* species resulted in 56 peaks corresponds to compounds among which 36 (64.3%) were isolated and characterized. Anthrones and chromones were found dominant compound classes accounted 23 compounds (65.7%). The most common compounds were aloin A/B followed with microdentin and aloinoside. In addition, a number of unique compounds like, 7-O-Methylaloesin, trihydroxy octadecenoic acid, 7-Hydroxy-8-O-methylaloin B, deacetylitoraloin, 6'-O-Coumaroyl aloesin, 7-O-Methyl-2'-feruloylaloesin, 1-hexanol-pentosyl hexoside, 1-O-methylplicalaloside, isorabichromone, and caffeoyl ester of aloesin have been isolated and characterized. The phytochemical characterization of *A. pirottae*, *A. megalacantha* subsp. *alticola*, *A. trichosantha* subsp. *longiflora* were resulted in unique characteristic compounds. The phytochemical variation was found significantly different ($P < .05$) between the two sub species of *Aloe megalacantha*, which could raise taxonomic discussion. These findings will assist for development of *Aloe* products by emerging pharmaceutical and cosmetic industries in Ethiopia through wide-scale cultivation in marginal and non-agricultural lands suitable for *Aloe* cultivation.

Keywords: *Aloe megalacantha*, *Aloe pirottae*, aloin, aloinoside, deacetylitoraloin, 7-O-Methylaloesin

1. INTRODUCTION

The phylogenetic tree of the [APG IV \(Byng et al., 2016\)](#) indicated that the genus *Aloe* (family: Asphodelaceae) is a member of the lily family that bears important multipurpose plants, such as onion, garlic and turnip families. Aloes like other members in the family have a number of bioactive compounds contribute profound values for humans ([Sebsebe and Nordal, 2010](#); [Cock, 2015](#)). However, research interests were focused on limited number of *Aloe* species like, *A. arborescens*, *A. barbadensis*, and *A. ferox*, which were known to be used by food processing, cosmetics, and pharmaceutical industries in many countries ([Oldfield, 2004](#); [Grace et al., 2009](#)). In recent years, the genus *Aloe* has attracted significant interest among consumers and researchers due to its diverse secondary metabolites of medicinal and cosmetic values extracted from leaves ([Chen et al., 2012](#); [Javed and Atta-Ur, 2014](#); [Bjorå et al., 2015](#); [Cardarelli et al., 2017](#)). A number of research findings conduction on the secondary metabolites from leaf and root extracts of some *Aloe* species have been revealed the possession of diverse bioactive compounds ([Reynolds, 1996](#); [Dagne et al., 2000](#); [Lucini et al., 2015](#); [Cardarelli et al., 2017](#)).

It is the leaf exudates that contain diverse phenolic compounds, which can be distinguished chromatographically ([Reynolds, 1985](#)). Currently more than 200 aloe compounds isolated belonging to different compound classes such as, anthraquinones, anthrones, chromones, coumarins, pyrones, alkaloids, glycoproteins, naphthalenes, steroids, volatile oils and flavonoids ([Reynolds, 1985](#); [Dagne et al., 2000](#); [Viljoen et al., 2001](#); [Cock, 2015](#); [Cardarelli et al., 2017](#); [Salehi et al., 2018](#)). In numerous pre-clinical and clinical studies, these *Aloe* compounds have exhibited various pharmacological activities and therapeutic effects due to the synergistic action of several phytochemicals compounds rather than from a single component ([Steenkamp and Stewart, 2007](#); [Lucini et al., 2015](#)). These classes of compounds have been shown to possess wound healing, anti-viral, anti-bacterial, anti-plasmodial or anti-malarial, anti-tumour, anti-ulcer, anti-diabetic, hypoglycaemic, anti-cancer, anti-inflammatory, anti-oxidant, and anti-hyperlipidemic activities ([Jia et al., 2008](#); [Grace et al., 2009](#); [Akaberi et al., 2016](#); [Radha and Laxmipriya, 2015](#); [Gelila et al., 2011](#); [Anwar et al., 2014](#); [Biruktawit et al., 2015](#); [Tibebu et al., 2018](#)). In addition, commercial preparations containing *Aloe* species include laxative drugs,

health drinks and tonics, after shaving gel, mouthwash and toothpaste, hair tonic and shampoo, and skin-moistening gel (Oldfield, 2004; Reynolds, 2005).

Some research results carried out on Ethiopian *Aloe* species indicated that the local communities make use of them for traditional health care system to treat a number of ailments (Anteneh *et al.*, 2012; Anteneh and Negussie, 2014), attributed to an enormous phytochemical products locked up in Aloes. Though comprehensive works are still lacking on the phytochemistry of Ethiopian aloes, the limited works done so far indicated that they possess diverse chemical constituents as well as unique types of compounds, especially the leaf exudates (Dagne *et al.*, 2000; Gelila *et al.*, 2011; Anwar *et al.*, 2014; Genet *et al.*, 2014; Mekdes *et al.*, 2015), which could have potential for pharmaceutical application compatible with the current commercialized *Aloe* species (Sisay *et al.*, 2013; Teshome, 2014; Damtew *et al.*, 2014, Yitagesu *et al.*, 2014; Biruktawit *et al.*, 2015). In view of the broad number of *Aloe* species in Ethiopia (46 identified and documented species with three subspecies, 67.3% are endemic), major gaps exist to recommend the gel and/or exudates of aloes for manufacturing application; this might be due to the limited knowledge of the phytochemical potential across these species. In this sense, the isolation, characterization, and quantification of bioactive compounds in *Aloe* species could allow the exploitation of enormous potential locked up in the Ethiopian *Aloe* species for the cosmetic, pharmaceutical, and food industries.

Therefore, the aim of this chapter was to analyze the phytochemistry of targeted *Aloe* species using ultra-high-performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS) and photodiode array detection (UV-PDA). Nowadays, this technique is among the best analytical tool to isolate, characterize, and quantify secondary metabolites in plants (Chen *et al.*, 2009; Cao *et al.*, 2009; Epriliati *et al.*, 2010; Lamuela-Raventós *et al.*, 2014), whose advantages are the rapid separation of compounds and the most accurate determination of the masses and the UV spectrums (Chen *et al.*, 2009; Lamuela-Raventós *et al.*, 2014). Therefore, using advanced techniques of UHPLC-MS and UV-DAD, isolation and characterization of compounds from the leaf exudates of targeted *Aloe* species have been analysed for identification of the potential Ethiopian *Aloe* species in industrial relevance and maximize their conservation.

2. MATERIALS AND METHODS

2.1. Chemicals

The major chemicals used are like, Acetonitrile (CH_3CN), chloroform/trichloromethane (CHCl_3), ethyl acetate/EtOAc ($\text{C}_4\text{H}_8\text{O}_2$), methanol/MeOH (CH_3OH), formic acid (CH_2O_2), and ultrapure water purified with a NaNo pure Diamond UV system (Barnstead International, Dubuque, IA, USA). All chemicals used for mobile phase were UHPLC analytical grade from Burdick & Jackson (Muskegon, MI, USA) and from Sigma-Aldrich (St. Louis, MO, USA). Standards used in optimization of UHPLC-MS/MS were also standard grades from Tshwane University of Technology (TUT), Department of Pharmaceutical Sciences laboratories in Pretoria, South Africa. The purity of reference compounds used as standards was higher than 95%.

2.2. Equipments

An ACQUITY ultra-highperformance liquid chromatography (UHPLC) coupled with mass spectrometry (MS) and ultraviolet-photodiode array detection (UV-DAD) system with Masslynx (version 4.2) software (Waters[®]) was used. This system consisting of the following components: ACQUITY UHPLC BEH C18 Column (150mm x 2.1 column-Ireland; particle packing size 1.7 μm); Agilent Technologies 7693 Autosampler; Waters Zspray; ACQUITY sample manager; ACQUITY PDA e λ detector (Waters Acquity PDA detector type was UPLC LG 500 nm, bandwidth 1.2 nm); and Isocratic Solvent Manager and QDa Detector (ESI $\pm$, cone: 15 V, capillary: 0.8kV, 150 to 850 m/z).

2.3. Sample Preparation

A portion of each *Aloe* exudate (250 μg) was dissolved in 1mL of 0.1% formic acid in 100% acetonitrile filtered through a 0.45 μm micropore membrane (PTFE, Waters Milford, MA, USA) before use. Later, it was injected into the UHPLC-PDA and ESI-Orbitrap-MS equipment with an injection volume of 1.00 μl .

2.4. UHPLC/ESI-MS-MS -DAD Procedure

Ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis coupled with ultraviolet-photodiode array detection (UV-DAD) were performed on a Waters® Acquity UHPLC system (Waters, Milford, MA, USA) equipped with electrospray ionization (ESI), photodiode array (PDA) detector/UV spectrum in the negative mode, an auto sampler, and column oven for identification and characterization of secondary metabolites in the target *Aloe* species. To achieve chromatograms with better resolution in short analysis time, the chromatographic conditions were optimized in the preliminary test (Chen *et al.*, 2009; Cao *et al.*, 2009; Epriliati *et al.*, 2010; Lamuela-Raventós *et al.*, 2014). The chromatographic separation was carried out using a Waters® Acquity UHPLC BEH C18 column; 2.1 mm x 150 mm; 1.7µm particle packing size (Waters-Ireland), which provide higher resolution and better efficiency than the conventional columns (Novakova *et al.*, 2006; Meier *et al.*, 2017).

The column temperature was set to 40 °C during operation and target sample temperature was 25 °C. A five-minute gradient program was optimized at a flow rate of 0.5 mL min⁻¹ with an injection volume of 5 µL. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow rate of 0.30 ml/min. The gradient parameters/elution were as follows: 00 min, 0.3 flow rate from 95% A and 5% B, 13,5 min, 0.3 flow rate from 60% A to 40% B, 14 min, flow rate 0.3, from 20% A to 80% B, 14.5 min, flow rate 0.3, from 95% A to 5% B (back to initial ratio). The injection volume was 1.0 µL (full-loop injection). Data was collected between 100 and 1500 m/z. The LC eluent was introduced directly into the electrospray ionization source without splitting the flow of the eluent. The chromatograms were recorded at wavelength of 287 nm while the UV spectra were monitored over a range of 400–200 nm.

2.5. Data Analysis

The chemometric data of the UHPLC-MS- diode array/UV spectrum data were analyzed using MassLynx (version 4.2) software. Peaks were identified by comparing retention times, pseudo molecular ions mass, and UV spectra as well as compared with some reference standards.

3. RESULTS

3.1. UHPLC-MS Chromatograms for Phytochemical Characterization

The phytochemical isolation from leaf exudates of 16 *Aloe* species resulted in about 56 compounds of secondary metabolites, in which 36 are identified/characterized (64.3%) and 21 were unidentified (35.7%) using UHPLC-MS and DAD UV spectrum methods (Table 1). The identified and characterized compounds belonging into nine compound classes i.e. anthraquinones, anthrones, chromones, coumarins, benzene, naphthalene and furan derivatives, flavonoid, oxilipin, pyrans and pyrones, and alcohol. The DAD UV spectrum were used to determine the compound classes whereas, the retention time, pseudomolecular ion (m/z) [M-H]⁻, and fragmentation ions were used for characterization of the isolated compounds. Anthrones and chromones were found the dominant compound classes accounted about 65.7% with 11 anthrones (31.4%) and 12 chromones (34.3%) of the identified compounds in the leaf exudates of the targeted *Aloe* species (Table 1).

Table 1. List of compounds and their classes isolated and characterized from the targeted *Aloe* species.

No.	Proposed structure	Compound class
1	Aloe-emodin	Anthraquinon
2	Aloe-emodin-8-O-glucoside (5-Hydroxyaloin-6'-acetate)	Anthraquinon
3	Aloin A	Anthron
4	Aloin B	Anthron
5	Aloinoside A	Anthron
6	Aloinoside B	Anthron
7	Deacetylaloin	Anthron
8	Homonataloin A	Anthron
9	Homonataloin B	Anthron
10	Microdantin A	Anthron
11	Microdantin B	Anthron
12	7-Hydroxy-8-O-methylaloin B	Anthron
13	Homonataloside A/B	Anthron
14	Plicataloside	Naphthalene derivatives
15	1-O-methylplicataloside	Naphthalene derivatives
16	7-O-Methylaloeresin A	Chromon
17	Isoaloeresin D	Chromon

18	Aloeresin A	Chromon
19	Aloeresin D, [M-H]- 555.1858 (m/z)	Chromon
20	Aloesin	Chromon
21	7-O-Methylaloesin	Chromon
22	6'-O-Coumaroyl aloesin	Chromon
23	Aloeresin D, [M-H]- 505.1332 (m/z)	Chromon
24	Aloeresin G	Chromon
25	Caffeoyl ester of aloesin	Chromon
26	Isorabichromone	Chromon
27	7-O-Methyl-2'-feruloylaloesin	Chromon
28	<i>p</i> -Coumaric acid derivatives	Coumarin
29	Dihydroisocoumarin	Coumarin
30	5,3'-Dihydroxy-6,7,4'-trimethoxyflavone(eupatorin)	Flavonoid
31	Kaempferol-3-O-hexosyl-O- pentoside	Flavonoid
32	Trihydroxy octadecenoic acid	Oxilipin
33	Aloenin	Pyrans and pyrones
34	3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- <i>b</i> -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one	Pyrans and pyrones
35	Aloenin B	Pyrans and pyrones
36	1-Hexanol-pentosylhexoside	Alcohol

The peak number, retention time, and molecular mass of the targeted *Aloe* species (*Aloe calidophila*, *A. harlana*, *A. mcloughlinii*, *A. megalacantha* subsp. *megalacantha*, *A. megalacantha* subsp. *alticola*, *A. otallensis*, *A. pirottae*, *A. pubescens*, *A. rivaie*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora*, and *A. yavellana*) are indicated on each peak of the UHPLC-MS chromatogram, arranged top to bottom (Figure 1-15). These isolated compounds per each peak were characterized by their retention time, pseudomolecular ion (m/z) [M-H]-, compound name, proposed chemical formula, and percentage composition in a given gram of dried aloe leaf exudate powder of the targeted *Aloe* species (Table 2-15). *Aloe pirottae* ranked first with 20 isolated compounds and followed with *A. secundiflora* and *A. tewoldei* with 15 compounds each and *A. harlana* and *A. mcloughlinii* each with 14 compounds. *A. calidophila* and *A. rivaie* has each with 13 compounds. The least number of compounds was isolated from *A. rugosifolia*, four compounds. Among the four compounds in *A. rugosifolia* 95 percent is constituted by a single compound plicataloside, which is a benzene, naphthalene and furan derivative. Unlike other *Aloe* species targeted in this study, the leaf exudate samples of

Aloe retrospiciens and *A. ruspoliana* collected and prepared in the same methods and conditions did not showed any compounds in all techniques followed and used in this study.

3.1.1. *Aloe calidophila* Reynolds

The UHPLC-MS chromatogram of *A. calidophila* has been resulted in 13 major peaks among which 11 compounds were identified and characterized (Figure 1). Only two peaks [M-H]⁻ ion at m/z 709.2128 and [M-H]⁻ ion at m/z 723.2317 were unidentified due to overlapped and unresolved peaks. The chromatogram peak 2 is characterized as anthraquinon (UV λ_{max} : 256, 285, 430 nm), peaks 4, 5, 6, 7, 11, and 12 are anthrones (UV λ_{max} : 268, 296, 355 nm), peaks 3 is pyran and pyron (UV λ_{max} : 244, 271, 323), peak 8 is flavonoid (UV λ_{max} : 255, 340 nm), and peaks 1 and 9 are coumarins (UV λ_{max} : 244, 271, 323) (Appendix IV).

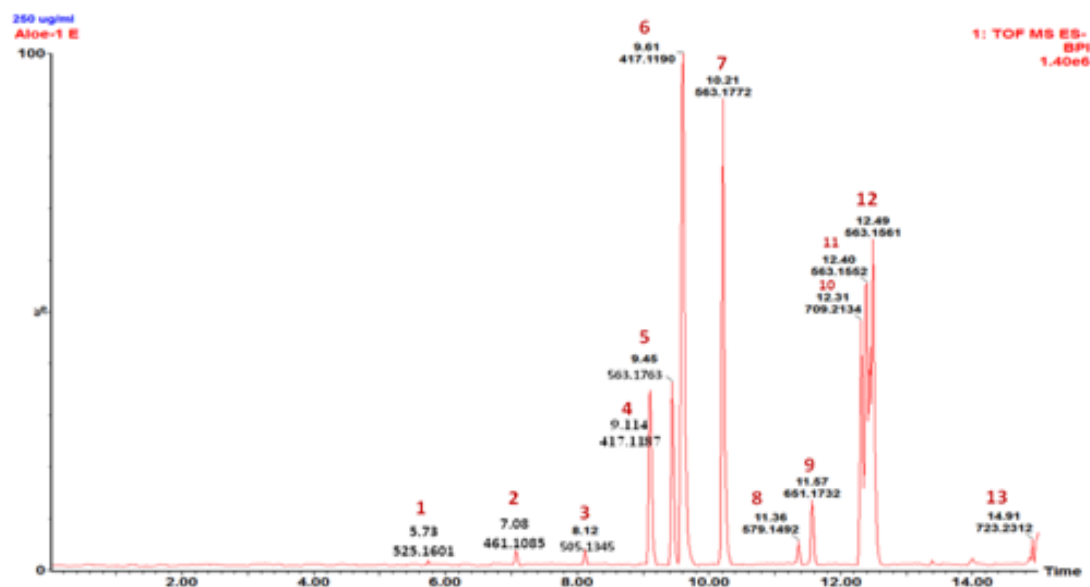


Figure 1. UHPLC-MS chromatogram of *Aloe calidophila* with peak number, retention time (Rt) in minute (Min), and pseudomolecular ion in m/z [M-H]⁻.

The anthrones were found dominant compound class in this species and no chromones were identified (Table 2).

Table 2. Isolated, characterized, and quantified compounds from the leaf exudate of *Aloe calidophila*.

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H] ⁻	Compounds	Chemical formula	Percentage
1	5.734	525.1601	Dihydroisocoumarin	C ₂₄ H ₂₉ O ₁₃	1
2	7.080	461.1085	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	2
3	8.120	505.1345	3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O-β-D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one	C ₂₄ H ₂₅ O ₁₂	-
4	9.114	417.1187	Aloin B	C ₂₁ H ₂₁ O ₉	9
5	9.466	563.1763	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	7
6	9.608	417.1190	Aloin A	C ₂₁ H ₂₁ O ₉	14
7	10.227	563.1782	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	16
8	11.340	579.1497	Kaempferol-3-O-hexosyl-O- pentoside	C ₃₀ H ₂₇ O ₁₂	-
9	11.568	651.1732	<i>p</i> -Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	-
10	12.329	709.2128	Unidentified	C ₃₆ H ₃₇ O ₁₅	-
11	12.505	563.1558	Microdontin B	C ₃₀ H ₂₇ O ₁₁	Both A
12	12.505	563.1561	Microdontin A	C ₃₀ H ₂₇ O ₁₁	& B 33
13	14.891	723.2317	Unidentified	C ₃₇ H ₃₉ O ₁₅	

3.1.2. *Aloe harlana* Reynolds

The UHPLC-MS chromatogram of *A. harlana* has been resulted in 14 major peaks among which 13 compounds were identified and characterized (Figure 2). Only one peak [M-H]⁻ ion at m/z 709.2110 was unidentified due to overlapped and unresolved peak. The chromatogram peaks 1 and 12 are coumarins (UV λ_{max}: 244, 271, 323), peak 2 is anthraquinon (UV λ_{max}: 256, 285, 430 nm), peaks 3 and 4 are pyrans and pyrones (UV λ_{max}: 244, 271, 323), peaks 5 and 10 are chromones (UV λ_{max}: 215, 234, 265, 297 nm), peaks 6,7,8,9, and 14 are anthrones (UV λ_{max}: 268, 296, 355 nm), and peak 11 is flavonoid (UV λ_{max}: 255, 340 nm) (Appendix I).

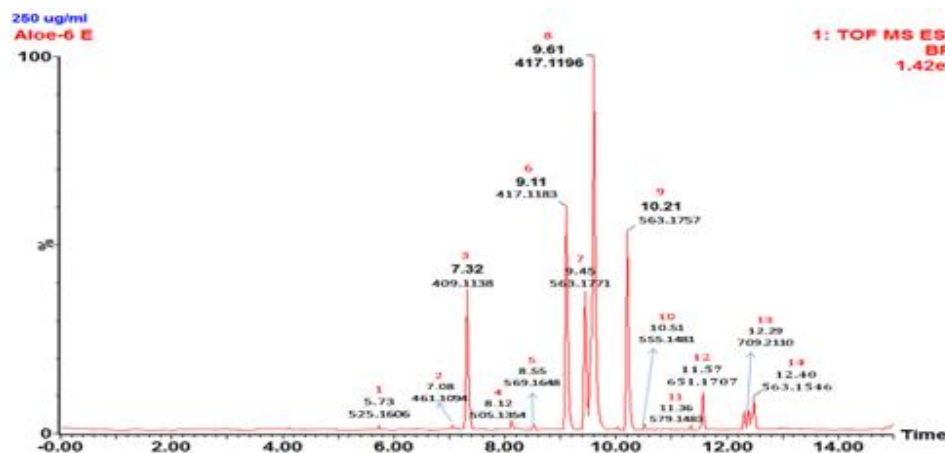


Figure 2. The UHPLC-MS chromatogram of *Aloe harlana* with peak number, retention time, and molecular ion in m/z

Six compound classes with 13 compounds were identified and characterized from this narrowly endemic *Aloe* species (Table 3) known only from "Harla village" near Dire Dawa town of eastern Ethiopia.

Table 3. Compounds isolated, characterized, and quantified from leaf exudate of *Aloe harlana*.

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H]-	Chemical formula	Compound	Percentage
1	5.734	525.1606	Dihydroisocoumarin	C ₂₄ H ₂₉ O ₁₃	1
2	7.08	461.1094	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	2
3	7.325	409.1138	Aloenin	C ₁₉ H ₂₁ O ₁₀	-
4	8.120	505.1354	3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one	C ₂₄ H ₂₅ O ₁₂	-
5	8.546	569.1648	Caffeoyl ester of aloesin	C ₂₉ H ₂₉ O ₁₂	-
6	9.114	417.1183	Aloin B	C ₂₁ H ₂₁ O ₉	5
7	9.449	563.1771	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	1
8	9.608	417.1196	Aloin A	C ₂₁ H ₂₁ O ₉	42
9	10.210	563.1757	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	10
10	10.506	555.1481	Aloeresin D	C ₂₈ H ₂₇ O ₁₂	1
11	11.358	579.1483	Kaempferol-3-O-hexosyl-O-pentoside	C ₃₀ H ₂₇ O ₁₂	-
12	11.568	651.1707	p-Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	-
13	12.295	709.2110	Unidentified	C ₃₆ H ₃₇ O ₁₅	-
14	12.398	563.1549	Microdantin B	C ₃₀ H ₂₇ O ₁₁	5

3.1.3. *Aloe mcloughlinii* Chris.

The UHPLC-MS chromatogram of *A. mcloughlinii* has been resulted in 14 major peaks among which 11 compounds were identified and characterized (Figure 3). Three peaks [M-H]⁻ ions at m/z 249.0760, 813.2274, and 709.2126 were unidentified due to overlapped and unresolved peaks. The chromatogram peaks 2 and 11 are coumarins (UV λ_{max} : 244, 271, 323), peaks 3, 4, and 12 are chromones (UV λ_{max} : 215, 234, 265, 297 nm), peaks 5, 6, 8, 13, and 14 are anthrones (UV λ_{max} : 268, 296, 355 nm), and 9 and 10 are flavonoid (UV λ_{max} : 255, 340 nm) (Appendix I).

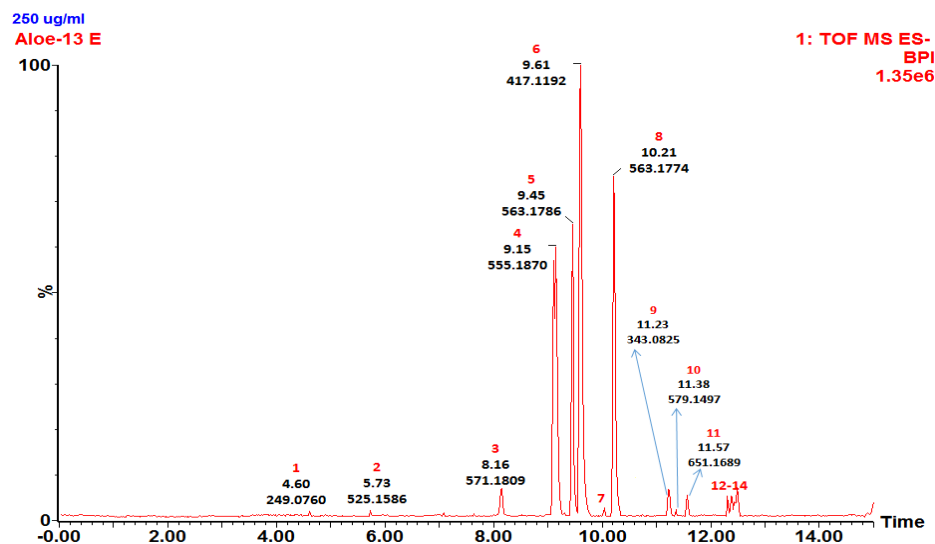


Figure 3. The UPLC-MS chromatogram of *Aloe mcloughlinii* with peak number, retention time, and molecular ion in m/z

Unique and new reports of varied compounds were isolated and characterized from this narrowly endemic *Aloe* species (Table 4), which has not been reported from this species so far.

Table 4. Compounds isolated, characterized, and quantified from leaf exudate of *Aloe mcloughlinii*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H]-	Compound	Chemical formula	Percent age
1	4.604	249.0760	Unidentified	C ₁₃ H ₁₃ O ₅	-
2	5.734	525.1586	Dihydroisocoumarin	C ₂₄ H ₂₉ O ₁₃	2
3	8.159	571.1809	Isorabichromone	C ₂₉ H ₃₁ O ₁₂	-
4	9.148	555.1870	Aloeresin D	C ₂₉ H ₃₁ O ₁₁	2
5	9.449	563.1786	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	6
6	9.608	417.1192	Aloin A	C ₂₁ H ₂₁ O ₉	24
7	10.051	813.2274	Unidentified	C ₃₉ H ₄₁ O ₁₉	-
8	10.21	563.1774	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	10
9	11.233	343.0825	5,3'-Dihydroxy-6,7,4'-trimethoxyflavone(eupatorin)	C ₁₈ H ₁₅ O ₇	-
10	11.375	579.1497	Kaempferol-3-O-hexosyl-O- pentoside	C ₃₀ H ₂₇ O ₁₂	-
11	11.568	651.1689	p-Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	-
12	12.329	709.2126	Unidentified	C ₃₆ H ₃₇ O ₁₅	-
13	12.36	563.1563	Microdantin B	C ₃₀ H ₂₇ O ₁₁	Both
14	12.488	563.1563	Microdantin A	C ₃₀ H ₂₇ O ₁₁	28

3.1.4. *Aloe megalacantha* Baker

This is the first study on the phytochemical analysis from the leaf exudate of the two sub species of *Aloe megalacantha* Baker. The two sub species, *A. megalacantha* subsp. *megalacantha* and *A. megalacantha* subsp. *alticola* showed much phytochemical variation. The later is an endemic

aloe sub species to Ethiopia restricted only to the east and west Hararge mountain chains. The UHPLC-MS chromatogram peaks 1, 4, 5, and 6 for *A. megalacantha* subsp. *megalacantha* showed $[M-H]^-$ ion at m/z : 461.1124 (Rt 7.06 min), 563.18 (Rt 9.45 min), 417.1216 (Rt 9.61 min), and 563.1807 (Rt 10.21 min) were characterized as aloe-emodin, aloinoside B, aloin A, and aloinoside A, respectively. These are the only compounds shared between these two sub species indicated on peaks 4, 7, 8, and 9 on the UHPLC-MS chromatogram for *A. megalacantha* subsp. *alticola*, which are the most common anthrones in most *Aloe* species (Figure 4).

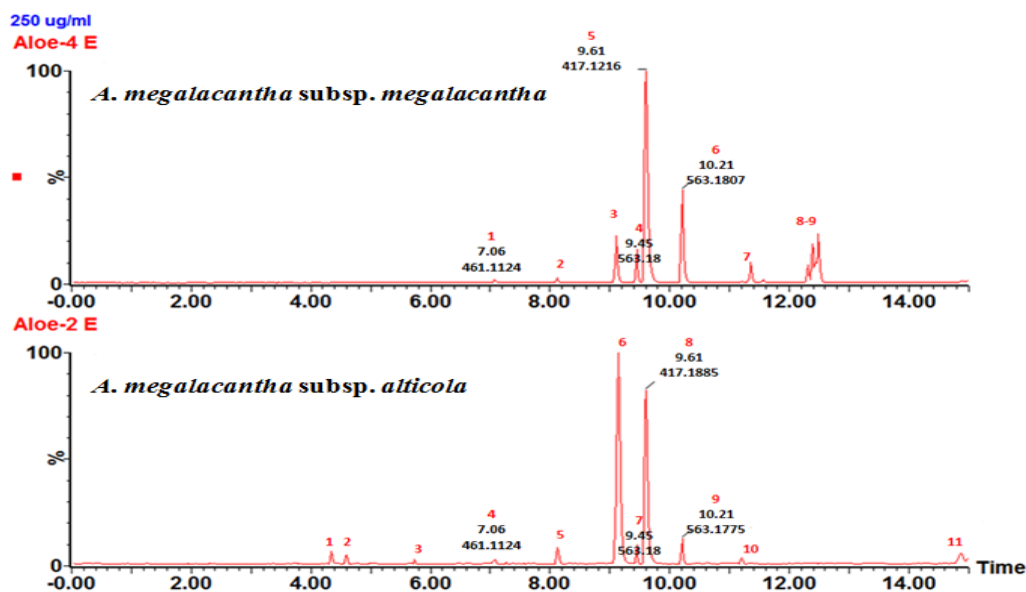


Figure 4. UHPLC-MS chromatogram variation of *A. megalacantha* Baker subsp. *megalacantha* and *A. megalacantha* Baker subsp. *alticola*

The UHPLC-MS chromatogram peaks 2, 3, 7, 8, and 9 of *A. megalacantha* subsp. *megalacantha* and peaks 1, 2, 3, 5, 6, 10, and 11 of *A. megalacantha* subsp. *alticola* are compounds, which are not shared among the two sub species. Rather than the similarity, the phytochemical profile of these two sub species was found significantly different ($P < .05$).

3.1.4.1. *Aloe megalacantha* Baker subsp. *megalacantha*

The UHPLC-MS chromatogram of *A. megalacantha* Baker subsp. *megalacantha* has been resulted in nine major peaks in which all have been identified and characterized (Figure 5). The UHPLC-MS chromatogram peak 1 is isolated as anthraquinon (UV $\lambda_{\max}$: 256, 285, 430 nm), peak 2 is chromon (UV $\lambda_{\max}$: 215, 234, 265, 297 nm), peaks 3, 4, 5, 6, 8 and 9 are anthrones (UV $\lambda_{\max}$: 268, 296, 355 nm), and peak 7 is flavonoid (UV $\lambda_{\max}$: 255, 340 nm).

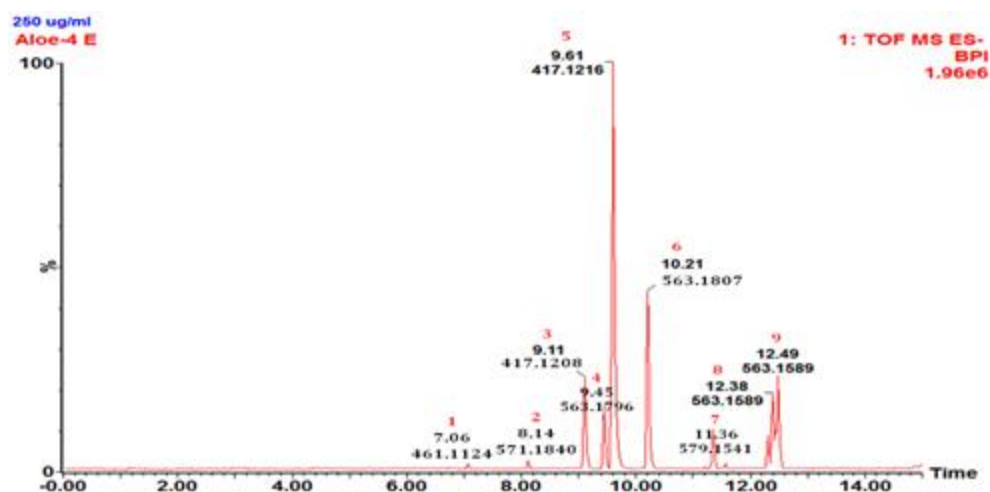


Figure 5. The UHPLC-MS chromatogram of *Aloe megalacantha* subsp. *megalacantha* with peak number, retention time, and molecular ion in m/z

Most of the bioactive compounds isolated and characterized from this sub species were anthrones except isorabaichromone and kaempferol-3-O-hexosyl-O- pentoside (Table 5).

Table 5. Compounds isolated, characterized, and quantified from the leaf exudate of *Aloe megalacantha* subsp. *megalacantha*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H]-	Compound	Chemical formula	Percentage
1	7.063	461.1124	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	1
2	8.142	571.1840	Isorabaichromone	C ₂₉ H ₃₁ O ₁₂	-
3	9.131	417.1208	Aloin B	C ₂₁ H ₂₁ O ₉	12
4	9.449	563.1796	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	5
5	9.608	417.1216	Aloin A	C ₂₁ H ₂₁ O ₉	28
6	10.227	563.1807	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	8
7	11.357	579.1541	Kaempferol-3-O-hexosyl-O- pentoside	C ₃₀ H ₂₇ O ₁₂	-
8	12.398	563.1602	Microdantin B	C ₃₀ H ₂₇ O ₁₁	Both
9	12.488	563.1589	Microdantin A	C ₃₀ H ₂₇ O ₁₁	32

3.1.4.2. *Aloe megalacantha* Baker subsp. *alticola* Baker

The UHPLC-MS chromatogram of *A. megalacantha* subsp. *alticola* has been resulted in 11 major peaks among which nine compounds were identified and characterized (Figure 6). Two peaks [M-H]- ions at m/z 607.1450 and 194.0818 were unidentified due to overlapped or unresolved peaks. The chromatogram peaks 3 is coumarin (UV λ_{max} : 244, 271, 323), peak 4 is isolated as anthraquinon (UV λ_{max} : 256, 285, 430 nm), peak 5 is pyrans and pyrones (UV λ_{max} :

244, 271, 323), peak 6 is chromon (UV λ_{max} : 215, 234, 265, 297 nm), and peaks 7, 8, and 9 are anthrones (UV λ_{max} : 268, 296, 355 nm) (Appendix I).

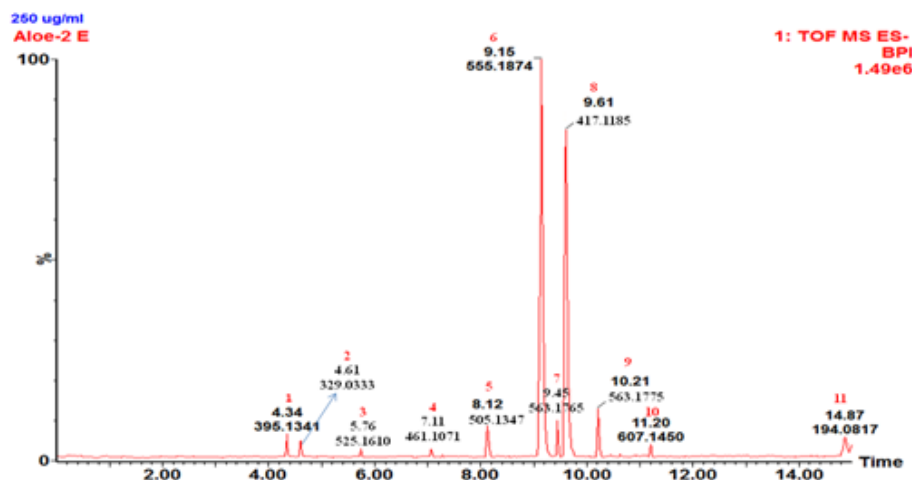


Figure 6. The UPLC-MS chromatogram of *Aloe megalacantha* subsp. *alticola*

Aloe megalacantha subsp. *alticola* was found to possess unique compounds of 1-Hexanol-pentosylhexoside, trihydroxy octadecenoic acid, dihydroisocoumarin, 3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one and aloeresin D (Table 6) that makes it different from the other sub species.

Table 6. Compounds isolated, characterized, and quantified from *Aloe megalacantha* subsp. *alticola*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H] ⁻	Compound types	Chemical formula	Percentage
1	4.342	395.1341	1-Hexanol-pentosylhexoside	C ₁₉ H ₂₃ O ₉	-
2	4.604	329.0333	Trihydroxy octadecenoic acid	C ₉ H ₁₃ O ₁₃	-
3	5.756	525.1610	Dihydroisocoumarin	C ₂₄ H ₂₉ O ₁₃	1
4	7.114	461.1071	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	4
5	8.120	505.1347	3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one	C ₂₄ H ₂₅ O ₁₂	-
6	9.131	555.1873	Aloeresin D	C ₂₉ H ₃₁ O ₁₁	-
7	9.449	563.1765	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	7
8	9.608	417.1185	Aloin A	C ₂₁ H ₂₁ O ₉	24
9	10.244	563.1775	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	24
10	11.198	607.1450	Unidentified	C ₃₁ H ₂₇ O ₁₃	-
11	14.891	194.0818	Unidentified	-	-

The types of compounds isolated and characterized make them much different that similar to the level of sub species. Even the common anthrones in most *Aloe* species, microdontin A and B, detected in *A. megalacantha* subsp. *megalacantha* were not isolated in this sub species.

3.1.5. *Aloe otallensis* Baker

The UHPLC-MS chromatogram of *A. otallensis* has been resulted in six major peaks but only three were identified and characterized (Figure 7). This species does not have the most common anthrons known in the genus *Aloe*. Rather benzene, naphthalene and furan derivatives are the dominant compound class in this species. The chromatogram peak 2 and 3 are benzene, naphthalene and furan derivatives (UV λ_{max} : 244, 271, 323) and peaks 6 is chromones (UV λ_{max} : 215, 234, 265, 297 nm).

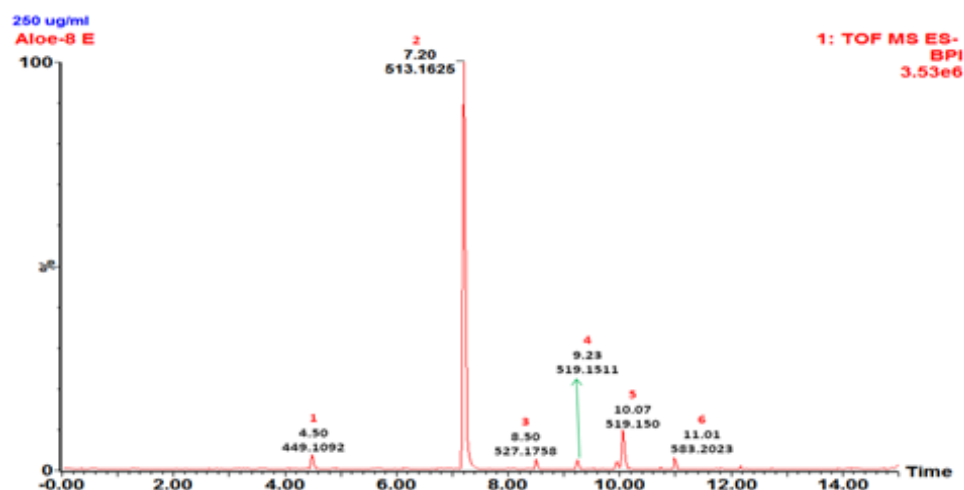


Figure 7. The UHPLC-MS chromatogram of *Aloe otallensis* with peak number, retention time, and molecular ion in m/z

Compared to the rest of *Aloe* species analyzed in this study, lower number of compounds were isolated and characterized from this species dominated by plicataloside (75%) (Table 7).

Table 7. Compounds isolated, characterized, and quantified from the leaf exudate of *Aloe otallensis*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H]-	Compound	Chemical formula	Percentage
1	4.501	449.1092	Unidentified	-	-
2	7.205	513.1625	Plicataloside	C ₂₃ H ₂₉ O ₁₃	75
3	8.495	527.1758	1-O-methylplicataloside	C ₂₄ H ₃₁ O ₁₃	-
4	9.234	519.1511	Unidentified	-	-
5	10.068	519.1500	Unidentified	-	-
6	11.005	583.2023	7-O-Methyl-2'-feruloylaloetin	C ₂₇ H ₃₅ O ₁₄	-

3.1.6. *Aloe pirottae* Berger

The UHPLC-MS chromatogram of *A. pirottae* has been resulted in 20 major peaks among which 15 compounds were identified and characterized (Figure 8). Five peaks [M-H]⁻ ions at m/z 555.1705, 541.1714, 709.2144, 665.1860, and 357.0982 were unidentified due to overlapped or unresolved peaks. The chromatogram peaks 1, 8, 10 and 14 are chromones (UV λ_{max} : 215, 234, 265, 297 nm), peak 4 is anthraquinon (UV λ_{max} : 256, 285, 430 nm), peak 5 is pyrans and pyrones (UV λ_{max} : 244, 271, 323), peaks 7 and 15 are coumarins (UV λ_{max} : 244, 271, 323), and peaks 9, 11, 12, 13, 17, and 18 are anthrones (UV λ_{max} : 268, 296, 355 nm).

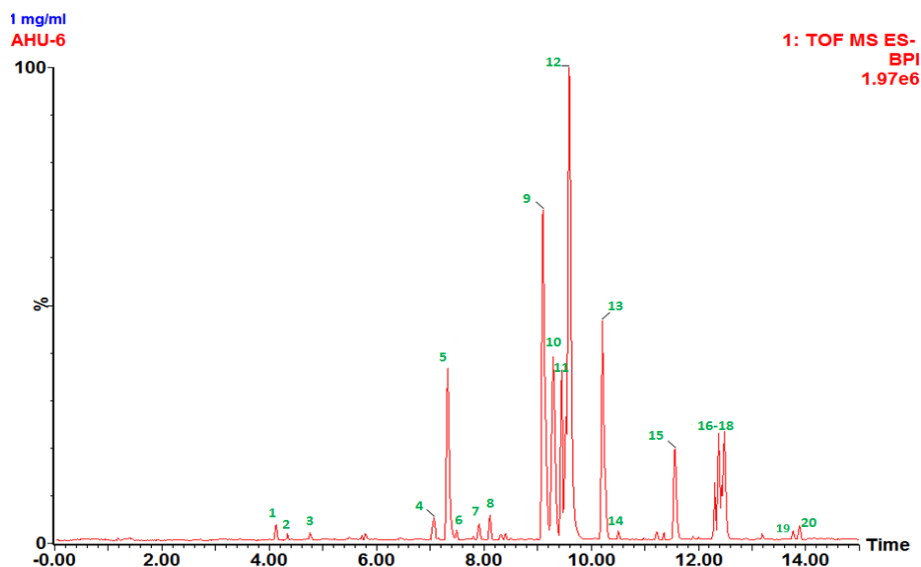


Figure 8. The UPLC-MS chromatogram of *A. pirottae* with peak number and retention time.

The number and diversity level of isolated and characterized compounds were much higher in this *Aloe* species (Table 8), which is the first phytochemical characterization of *A. pirottae*.

Table 8. Compounds isolated, characterized, and quantified from *Aloe pirottae*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H] ⁻	Compound	Chemical formula	Percent age
1	4.127	393.1180	Aloesin	C ₁₉ H ₂₁ O ₉	
2	4.342	395.1328	1-Hexanol-pentosylhexoside	C ₁₉ H ₂₃ O ₉	
3	4.763	555.1705	Unidentified	C ₂₅ H ₃₁ O ₁₄	
4	7.063	461.1107	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	5
5	7.325	409.1131	Aloenin	C ₁₉ H ₂₁ O ₁₀	
6	7.467	541.1714	Unidentified	C ₂₈ H ₂₉ O ₁₁	
7	7.893	539.1547	6'-O-Coumaroyl aloesin	C ₂₈ H ₃₁ O ₁₂	-
8	8.142	571.1814	Isorabaichromone	C ₂₉ H ₂₇ O ₁₁	
9	9.114	417.1188	Aloin B	C ₂₁ H ₂₁ O ₉	12

10	9.29	555.1858	Aloeresin D	C ₂₉ H ₃₁ O ₁₁	8
11	9.449	563.1765	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	4
12	9.608	417.1188	Aloin A	C ₂₁ H ₂₁ O ₉	34
13	10.244	563.1781	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	10
14	10.528	555.1502	Aloeresin D	C ₂₈ H ₂₇ O ₁₂	
15	11.568	651.1724	p-Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	
16	12.329	709.2144	Unidentified	C ₃₆ H ₃₇ O ₁₅	
17	12.39	563.1550	Microdantin B	C ₃₀ H ₂₇ O ₁₁	Both
18	12.46	563.1560	Microdantin A	C ₃₀ H ₂₇ O ₁₁	18
19	13.778	665.1860	Unidentified	C ₃₄ H ₃₃ O ₁₄	
20	13.885	357.0982	Unidentified	C ₁₉ H ₁₇ O ₇	

3.1.7. *Aloe pubescens* Reynolds

The UHPLC-MS chromatogram of *A. pubescens* has resulted in 11 major peaks among which nine compounds were identified and characterized (Figure 9). Two peaks [M-H]⁻ ions at m/z 489.1763 and 491.1912 were unidentified due to overlapped or unresolved peaks. The chromatogram peaks 1, 2, and 11 are chromones (UV λ_{max} : 215, 234, 265, 297 nm), peak 4 is anthraquinone (UV λ_{max} : 256, 285, 430 nm), and peaks 3, 5, 6, 7, and 8 are anthrones (UV λ_{max} : 268, 296, 355 nm).

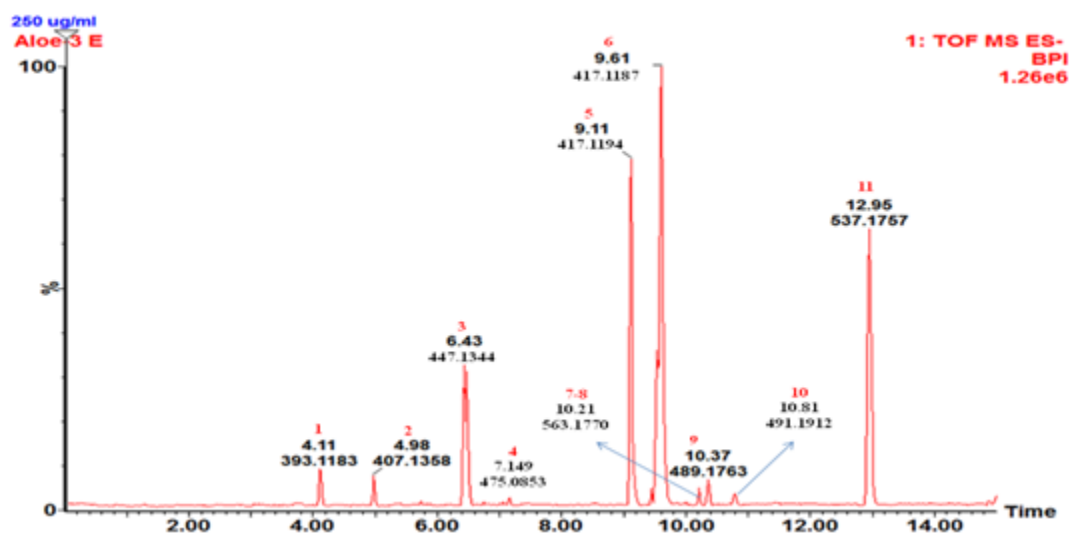


Figure 9. The UPLC-MS chromatogram of *Aloe pubescens* with peak number, retention time, and molecular ion in m/z

Like common in most aloe species, the anthrones and chromones are the dominant compound classes in this species (Table 9).

Table 9. Compounds isolated, characterized, and quantified from *Aloe pubescens* with their retention time (RT), molecular mass, chemical formula, and percentage composition

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H] ⁻	Compound	Chemical formula	Percentage
1	4.110	393.1183	Aloesin	C ₁₉ H ₂₁ O ₉	-
2	4.961	407.1344	7-O-Methylaloesin	C ₂₀ H ₂₃ O ₉	-
3	6.444	447.1284	7-Hydroxy-8-O-methylaloin B	C ₂₂ H ₂₃ O ₁₀	-
4	7.149	475.0853	Aloe-emodin-8-O-glucoside (5-Hydroxyaloin-6'-acetate)	C ₂₂ H ₁₉ O ₁₂	-
5	9.096	417.1194	Aloin B	C ₂₁ H ₂₁ O ₉	14
6	9.625	417.1187	Aloin A	C ₂₁ H ₂₁ O ₉	16
7	10.210	563.1770	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	1
8	10.227	563.1744	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	1
9	10.369	489.1763	Unidentified	C ₂₅ H ₂₉ O ₁₀	-
10	10.807	491.1912	Unidentified	C ₂₅ H ₃₁ O ₁₀	-
11	12.948	537.1757	Aloeresin G	C ₂₉ H ₂₉ O ₁₀	26

3.1.8. *Aloe rivae* Baker

The UHPLC-MS chromatogram of *A. rivae* has resulted in 13 major peaks among which nine compounds were identified and characterized (Figure 10). Four peaks [M-H]⁻ ions at m/z 725.2055, 709.2132, 679.2013 and 679.2007 were unidentified due to overlapped or unresolved peaks. The chromatogram peak 1 is pyran and pyron (UV λ_{max} : 244, 271, 323), peaks 2, 3, 4, 5, 6, 10, and 11 are anthrones (UV λ_{max} : 268, 296, 355 nm), and peak 7 is flavonoid (UV λ_{max} : 255, 340 nm).

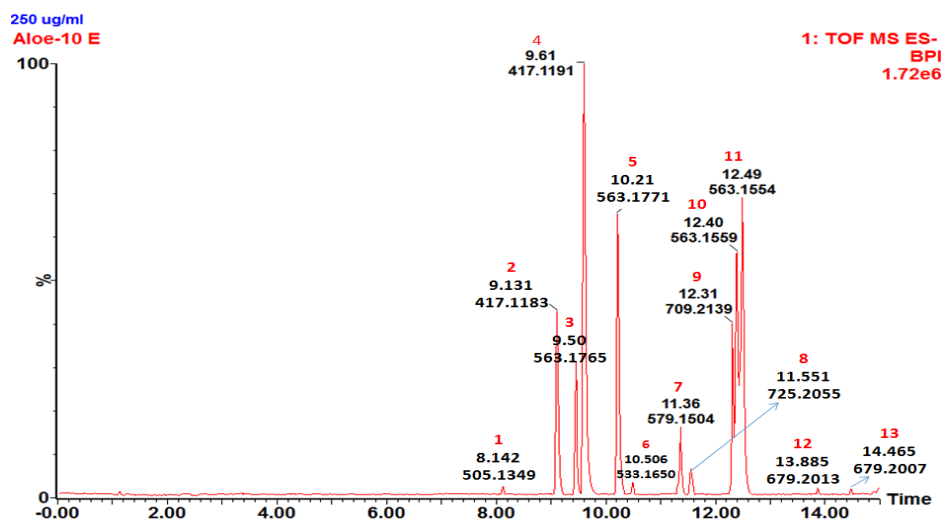


Figure 10. The UPLC-MS chromatogram of *Aloe rivae* with peak number, retention time, and molecular ion in m/z

This is stemless and non-spotted *Aloe* species possess phytochemical profile dominated by anthrones and no chromones at all (Table 10).

Table 10. Compounds isolated, characterized, and quantified from the leaf exudate of *Aloe riva*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H] ⁻	Compound	Chemical formula	Percentage
1	8.142	505.1349	3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl)methyl-2(1H)-benzopyran-1-one	C ₂₄ H ₂₅ O ₁₂	-
2	9.131	417.1183	Aloin B	C ₂₁ H ₂₁ O ₉	6
3	9.50	563.1765	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	4
4	9.608	417.1191	Aloin A	C ₂₁ H ₂₁ O ₉	25
5	10.21	563.1771	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	11
6	10.506	533.1650	Deacetylaloin	C ₂₆ H ₂₉ O ₁₂	-
7	11.375	579.1505	Kaempferol-3-O-hexosyl-O-pentoside	C ₃₀ H ₂₇ O ₁₂	-
8	11.551	725.2055	Unidentified	C ₃₆ H ₃₇ O ₁₆	-
9	12.346	709.2132	Unidentified	C ₃₆ H ₃₇ O ₁₅	-
10	12.380	563.1559	Microdantin B	C ₃₀ H ₂₇ O ₁₁	12
11	12.488	563.1553	Microdantin A	C ₃₀ H ₂₇ O ₁₁	16
12	13.885	679.2013	Unidentified	C ₃₅ H ₃₅ O ₁₄	-
13	14.465	679.2007	Unidentified	C ₃₅ H ₃₅ O ₁₄	-

3.1.9. *Aloe rugosifolia* Gilbert & Sebsebe

The least number of peaks were detected from *A. rugosifolia* and three compounds were isolated and characterized (Figure 11). The chromatogram peak 1 and 2 are benzene, naphthalene and furan derivatives (UV λ_{max} : 244, 271, 323) and peaks 4 is chromones (UV λ_{max} : 215, 234, 265, 297 nm).

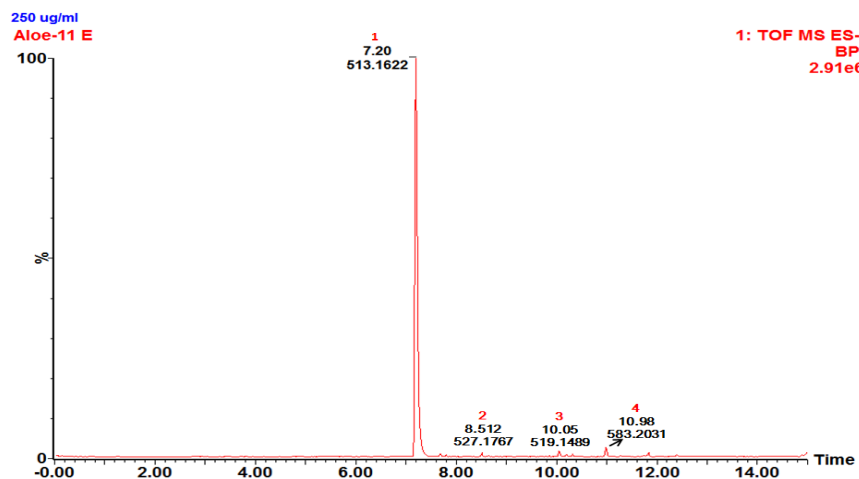


Figure 11. The UHPLC-MS chromatogram of *A. rugosifolia* with peak number, retention time, and molecular ion in m/z

Like *Aloe otallensis*, this species does not have anthrones. Rather benzene, naphthalene and furan derivatives are the dominant compound class in this species in which a single bioactive compound plicataloside accounted for 95% of the dried leaf exudate (Table 11).

Table 11. Compounds isolated, characterized, and quantified from the leaf exudate of *Aloe rugosifolia* with their retention time (RT), molecular mass, and chemical formula

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H] ⁻	Compound	Chemical formula	Percent age
1	7.205	513.1622	Plicataloside	C ₂₃ H ₂₉ O ₁₃	95
2	8.512	527.1767	1-O-methylplicataloside	C ₂₄ H ₃₁ O ₁₃	-
3	10.051	519.1489	Unidentified	-	-
4	10.983	583.2031	7-O-Methyl-2'-feruloylaloetin	C ₂₇ H ₃₅ O ₁₄	-

3.1.10. *Aloe secundiflora* Engler

The UPLC-MS chromatogram of *Aloe secundiflora* has resulted in 15 major peaks among which 11 compounds were identified and characterized (Figure 12). Four peaks [M-H]⁻ ions at m/z 409.1134, 509.1676, 863.2368, and 863.2405 were unidentified due to overlapped or unresolved peaks. The chromatogram peaks 2 and 15 are coumarins (UV λ_{max} : 244, 271, 323), peak 3 is anthraquinon (UV λ_{max} : 256, 285, 430 nm), peaks 4 and 6 are pyrans and pyrones (UV λ_{max} : 244, 271, 323), peaks 7, 8, and 12 are chromones (UV λ_{max} : 215, 234, 265, 297 nm), and peaks 9, 10, and 11 are anthrones (UV λ_{max} : 268, 296, 355 nm)

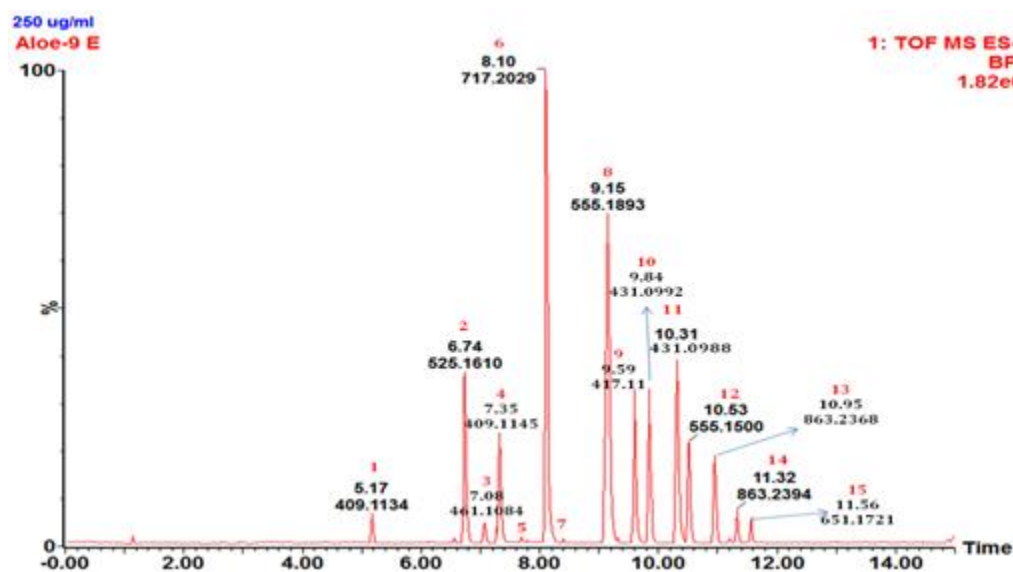


Figure 12. The UHPLC-MS chromatogram of *Aloe secundiflora* with peak number, retention time, and molecular ion in m/z

Higher numbers of compounds were isolated and characterized from this species for the first time (Table 12).

Table 12. Compounds isolated, characterized, and quantified from the leaf exudate of *Aloe secundiflora*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H]-	Compound	Chemical formula	Percentage
1	5.171	409.1134	Unidentified	C ₁₉ H ₂₁ O ₁₀	
2	6.745	525.1609	Dihydroisocoumarin	C ₂₄ H ₂₉ O ₁₃	1
3	7.080	461.1084	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	
4	7.364	409.1145	Aloenin	C ₁₉ H ₂₁ O ₁₀	1
5	7.716	509.1676	Unidentified	C ₂₄ H ₂₉ O ₁₂	
6	8.103	717.2029	Aloenin B	C ₃₄ H ₃₇ O ₁₇	-
7	8.12	505.1347	6'-Malonylnatalion	C ₂₄ H ₂₅ O ₁₂	
8	9.148	555.1893	Aloeresin D	C ₂₉ H ₃₁ O ₁₁	16
9	9.591	417.1197	Aloin B	C ₂₁ H ₂₁ O ₉	13
10	9.853	431.0992	Homonataloin B	C ₂₁ H ₁₉ O ₁₀	12
11	10.330	431.0988	Homonataloin A	C ₂₁ H ₁₉ O ₁₀	20
12	10.506	555.1509	Isoaloeresin D	C ₂₈ H ₂₇ O ₁₂	-
13	10.949	863.2368	Unidentified	C ₄₃ H ₄₃ O ₁₉	-
14	11.301	863.2405	Unidentified	C ₄₃ H ₄₃ O ₁₉	-
15	11.585	651.1721	p-Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	-

3.1.11. *Aloe tewoldei* Gilbert & Sebsebe

The UHPLC-MS chromatogram of *A. tewoldei* has resulted in 15 major peaks among which 12 compounds were identified and characterized (Figure 13). Three peaks [M-H]- ions at m/z 249.0763, 231.0669, and 709.2147 were unidentified due to overlapped or unresolved peaks. The chromatogram peak 3 is anthraquinon (UV λ_{max} : 256, 285, 430 nm), peak 4 is pyran and pyron (UV λ_{max} : 244, 271, 323), peaks 5, 6, 7, 8, 14, and 15 are anthrones (UV λ_{max} : 268, 296, 355 nm), peaks 9 and 10 are Flavonoid (UV λ_{max} : 244, 271, 323), and peak 11 and 12 are coumarins (UV λ_{max} : 244, 271, 323).

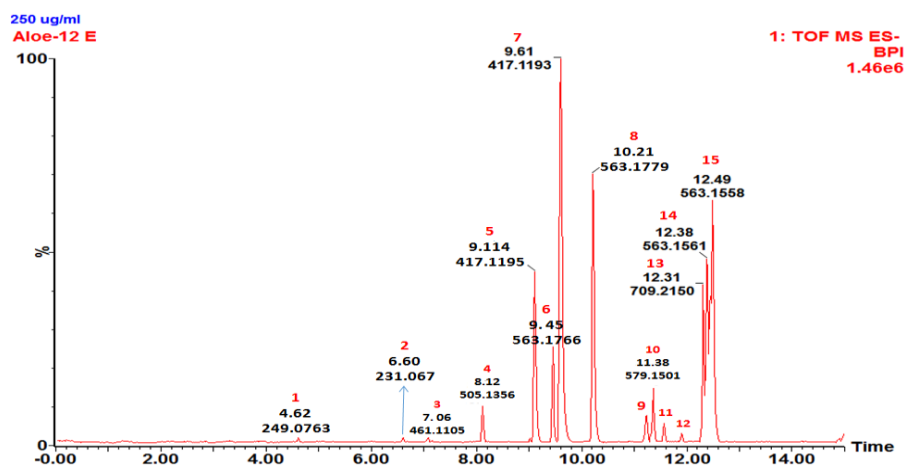


Figure 13. UPLC-MS chromatogram of *A. tewoldei*

This endemic *Aloe* species has been found to possess higher number of compounds dominated by anthrones (Table 13). The *p*-Coumaric acid derivatives, 5,3'-Dihydroxy-6,7,4'-trimethoxyflavone(eupatorin), and kaempferol-3-O-hexosyl-O- pentoside are not so far reported from Ethiopian.

Table 13. Compounds isolated, characterized, and quantified from the leaf exudate of *Aloe tewoldei*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H]-	Compound	Chemical formula	Percent age
1	4.621	249.0763	Unidentified	C ₁₃ H ₁₃ O ₅	-
2	6.603	231.0669	Unidentified	C ₁₃ H ₁₁ O ₄	-
3	7.063	461.1105	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	4
4	8.12	505.1356	3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O-β-D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one	C ₂₄ H ₂₅ O ₁₂	-
5	9.114	417.1195	Aloin B	C ₂₁ H ₂₁ O ₉	6
6	9.449	563.1766	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	4
7	9.608	417.1193	Aloin A	C ₂₁ H ₂₁ O ₉	26
8	10.21	563.1779	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	11
9	11.233	343.0811	5,3'-Dihydroxy-6,7,4'-trimethoxyflavone(eupatorin)	C ₁₈ H ₁₅ O ₇	-
10	11.375	579.1501	Kaempferol-3-O-hexosyl-O-pentoside	C ₃₀ H ₂₇ O ₁₂	-
11	11.568	651.1706	<i>p</i> -Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₂	-
12	11.92	651.1706	<i>p</i> -Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₂	-
13	12.329	709.2147	Unidentified	-	-
14	12.38	563.1561	Microdantin B	C ₃₀ H ₂₇ O ₁₁	12
15	12.488	563.1558	Microdantin A	C ₃₀ H ₂₇ O ₁₁	16

3.1.12. *Aloe trichosantha* subsp. *longiflora* Gilbert & Sebsebe

The UHPLC-MS chromatogram of *A. trichosantha* subsp. *longiflora* has resulted in 10 major peaks among which eight compounds were identified and characterized (Figure 14). Two peaks [M-H]⁻ ions at m/z 375.1092 and 607.1448 were unidentified due to overlapped or unresolved peaks. The chromatogram peaks 1 and 5 are chromones (UV λ_{max} : 215, 234, 265, 297 nm), peaks 2, 4, and 6 are anthrones (UV λ_{max} : 268, 296, 355 nm), and peak 3, 9, and 10 are coumarins (UV λ_{max} : 244, 271, 323).

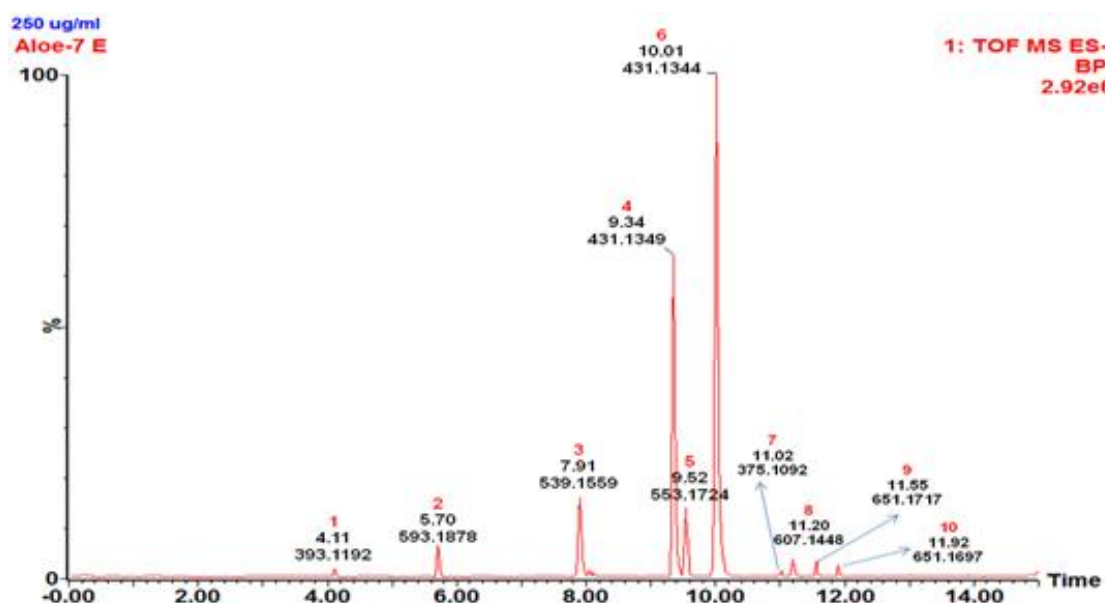


Figure 14. The UHPLC-MS chromatogram of *Aloe trichosantha* subsp. *longiflora*

Unlike most *Aloe* species, this sub species was found free from the marker compound of aloe known as Aloin, aloinoside, and microdentin (Table 14).

Table 14. Compounds isolated, characterized, and quantified from the leaf exudate of *A. trichosantha* subsp. *longiflora* with their retention time (RT), molecular mass, chemical formula, and percentage composition for some compounds.

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H] ⁻	Compound	Chemical formula	Percentage
1	4.110	393.1192	Aloesin	C ₁₉ H ₂₁ O ₉	-
2	5.700	593.1878	Homonataloside A/B	C ₂₈ H ₃₃ O ₁₄	4
3	7.910	539.1559	6'-O-Coumaroyl aloesin	C ₂₈ H ₂₇ O ₁₁	18
4	9.358	431.1349	Homonataloin B	C ₂₁ H ₁₉ O ₁₀	16
5	9.552	553.1724	7-O-Methylaloeresin A	C ₂₉ H ₂₉ O ₁₁	6
6	10.012	431.1344	Homonataloin A	C ₂₁ H ₁₉ O ₁₀	28
7	11.022	375.1092	Unidentified	C ₁₉ H ₁₉ O ₈	-
8	11.198	607.1448	Unidentified	C ₃₁ H ₂₇ O ₁₃	-
9	11.551	651.1717	p-Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	-
10	11.920	651.1697	p-Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	-

3.1.13. *Aloe yavellana* Reynolds

The UHPLC-MS chromatogram of *Aloe yavellana* has resulted in 12 major peaks among which 10 compounds were identified and characterized (Figure 15). Two peaks at m/z 709.2131 and 725.2047 were unidentified due to overlapped or unresolved peaks. The chromatogram peaks 1 is anthraquinon (UV λ_{max} : 256, 285, 430 nm), peak 2 is chromon (UV λ_{max} : 215, 234, 265, 297 nm), peaks 3, 4, 5, 6, 11, and 12 are anthrones (UV λ_{max} : 268, 296, 355 nm), peak 8 is flavonoid (UV λ_{max} : 244, 271, 323), and peak 9 is coumarin (UV λ_{max} : 244, 271, 323).

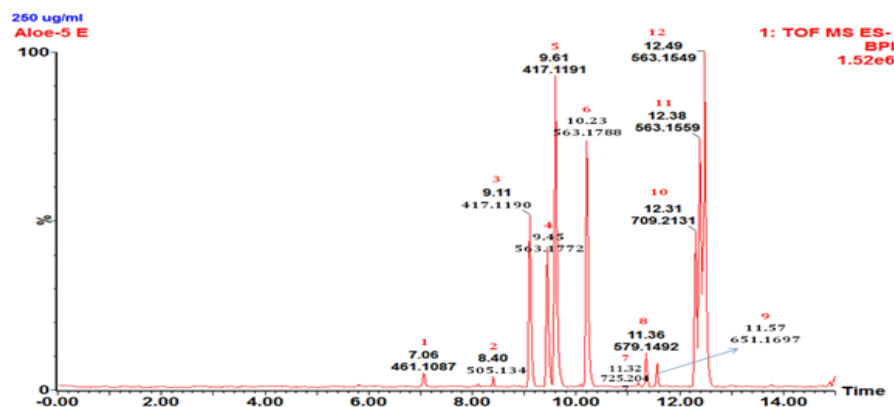


Figure 15. The UPLC-MS chromatogram of *A. yavellana* with peak number, retention time, and molecular ion in m/z

Like most *Aloe* species covered in this study, anthrones were found common bioactive compounds isolated and characterized from this narrowly endemic *Aloe* species (Table 15).

Table 15. Compounds isolated, characterized, and quantified from the leaf exudate of *Aloe yavellana*

Peak	Rt (Min)	Pseudomolecular ion (m/z) [M-H]-	Compound types	Chemical formula	Percentage
1	7.080	461.1086	Aloe-emodin	C ₂₂ H ₂₁ O ₁₁	1
2	8.387	505.1342	Dihydroisocoumarin	C ₂₄ H ₂₉ O ₁₃	4
3	9.114	417.1190	Aloin B	C ₂₁ H ₂₁ O ₉	8
4	9.449	563.1772	Aloinoside B	C ₂₇ H ₃₁ O ₁₃	8
5	9.608	417.1191	Aloin A	C ₂₁ H ₂₁ O ₉	18
6	10.227	563.1788	Aloinoside A	C ₂₇ H ₃₁ O ₁₃	12
7	11.323	725.2047	Unidentified	C ₃₆ H ₃₇ O ₁₆	-
8	11.357	579.1491	Kaempferol-3-O-hexosyl-O-pentoside	C ₃₀ H ₂₇ O ₁₂	-
9	11.568	651.1697	p-Coumaric acid derivatives	C ₃₃ H ₃₁ O ₁₄	-
10	12.312	709.2131	Unidentified	C ₃₆ H ₃₇ O ₁₅	-
11	12.380	563.1558	Microdantin B	C ₃₀ H ₂₇ O ₁₁	Both
12	12.488	563.1548	Microdantin A	C ₃₀ H ₂₇ O ₁₁	42

3.1.14. *Aloe retrospiciens* Reynolds & Bally and *Aloe ruspoliana* Baker

The HPTLC, UHPLC-MS and DAD UV spectrum techniques used in this study did not detect any bands, masses, and UV spectrums from the sticky solid and unpleasant smell leaf exudates of these two *Aloe* species. Therefore, no bioactive compounds were isolated and characterized from *Aloe retrospiciens* and *Aloe ruspoliana* using this advanced techniques HPTLC, UHPLC-MS and DAD UV spectrum (Figure 16).

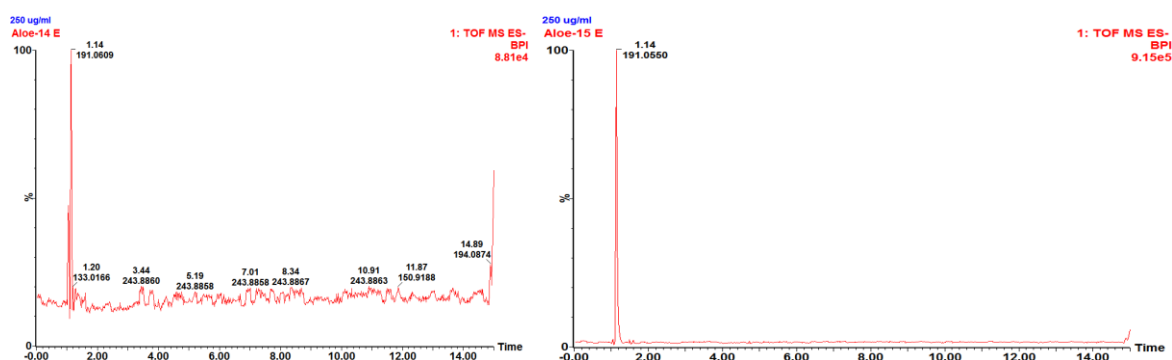


Figure 16. the UHPLC-MS Chromatogram of *Aloe retrospiciens* (Aloe-14E) and *Aloe ruspoliana* (Aloe-15E)

4. DISCUSSION

4.1. UHPLC-MS Chromatograms for Phytochemical Characterization

Most of the compounds isolated in this study belong into the anthrones and chromones compound classes. This supports the findings of studies (Reynolds, T., 1985; Dagne *et al.*, 2000; Viljoen *et al.*, 2001). Similarly, reports indicated that around 20 chemical constituents, including aloin A and B, aloinoside A and B, aloesin, plicataloside, microdontin A and B, and aloe emodin were isolated as principal *Aloe* exudate components through liquid chromatography mass spectrometry (LC-ESI/ MS/MS) (Cardarelli *et al.*, 2017; Salehi *et al.*, 2018). Other classes like, anthraquinones, pre-anthraquinones, benzene and naphthalene derivatives, coumarins, phenyl pyrones, and alkaloids are sporadically detected in *Aloe* leaves (Viljoen, 1999). A number of these sporadic classes of compounds were also detected in this study. Some unique and new compounds, which have not been reported so far from Ethiopian *Aloe* species such as 7-O-Methylaloesin, trihydroxy octadecenoic acid, 7-Hydroxy-8-O-methylaloin B, deacetylaloin, 6'-O-Coumaroyl aloesin, 7-O-Methyl-2'-feruloylaloesin, 1-hexanol-pentosyl hexoside, 1-O-methylplicataloside, caffeoyl ester of aloesin, isorabichromone, and aloenin B were isolated from some of the target *Aloe* species in this study. Though comprehensive works were lacking on the phytochemistry of Ethiopian Aloes, the limited works done so far on some Ethiopian *Aloe* species indicated that they possess diverse chemical constituents as well as unique types of compounds (Dagne *et al.*, 2000; Anwar *et al.*, 2014).

4.1.1. *Aloe calidophila* Reynolds

Anthrones are the dominant compound class in this species in which microdontin A and B together accounted the higher percentage followed by aloinoside A and aloin A. The high quantities of microdontins (A and B) resulted in unresolved peaks (Peaks 10-12). The leaf exudate from *A. boscaawenii* of Tanzania has shown an incomplete separation of the peak with microdontins due to the high quantities of these two isomer anthrones (Viljoen *et al.*, 2001) but the co-injection with a microdontin standard confirmed that the peaks correspond to microdontin B and A, respectively. In some species of *Aloe*, like *A. calidophila*, *A. boscaawenii*, *A. pirottae*, *A. riva*, *A. tewoldei* and *Aloe yavellana*, the third peak in the series displays the

same UV spectrum properties, i.e. [M-H]⁻ ion at m/z 709.2128 with Rt 12.329 could not be able to be characterized in this study. Among the compounds reported in this study, dihydroisocoumaringlucoside, aloin A/B, aloinoside A/B, and microdontin A/B were isolated and characterized using HPLC, preparative TLC, ¹³C-NMR, and MS methods from the leaf exudate of this species (Abeje *et al.*, 2014). Those isolated compounds showed strong anti-leishmanial activity (against *L. aethiopica* and *L. major*) and aloinoside was reported as better to arrest *L. aethiopica* and *L. major* activity than the standard drugs for Leishmaniasis (Abeje *et al.*, 2014). Additional compounds like, 3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one (feralolide-3'-O- β -D-glucopyranoside), aloe-emodin, kaempferol-3-O-hexosyl-O- pentoside, and *p*-Coumaric acid derivatives were isolated and characterized from *A. calidophila* for the first time in this study. Recently, feralolide-3'-O- β -D-glucopyranoside was isolated from the 70% EtOH extract of flowers of *Aloe arborescens* (Kurizaki *et al.*, 2019), which has been previously reported from the leaf exudate of *A. hildebrandtii* (Veitch *et al.*, 1994).

4.1.2. *Aloe harlana* Reynolds

The highest number of compounds were identified and characterized from this *Aloe* species. The anthrones like, aloin A/B, aloinoside A/B, and microdontin A/B; and chromone (7-O-methylaloeresin A) have been reported from *A. harlana* leaf exudate (Gelila *et al.*, 2011). Unlike previous reports, microdontin A and 7-O-methylaloeresin A were not detected in this study. However, the rest of the compounds like, dihydroisocoumarin, aloe-emodin, aloenin, 3,4-dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one, caffeoyl ester of aloesin, aloeresin D, kaempferol-3-O-hexosyl-O- pentoside, and *p*-coumaric acid derivatives were new reports in this study from this narrowly endemic *Aloe* species. The pharmacological study done by Gelila *et al.* (2011) indicated that the exudate and those isolated compounds of *A. harlana* possess promising antimicrobial activity particularly against the gram-negative bacterial strains such as *Escherichia coli*, *Salmonella typhi* and *Vibrio cholerae*. Aloin A (42%) is the highest concentration in the leaf exudate of this species among *Aloe* species so far reported. The presence of aloin has been reported in nearly 100 species of *Aloe*, usually at relatively high levels of 10–20 % in concentration (Dagne *et al.*, 2000). The amount of

aloin is also extremely variable among different species and highly depends on the growing conditions (Wamer *et al.*, 2003). Even with this variability, this compound has been reported to represent up to 30% of the dry weight of aloe latex/exudate (Rebecca *et al.*, 2003). *A. harlana* could be the good source of aloin compared to any other *Aloe* species reported so far.

Aloe harlana was reported to be used for a number of ailments in the traditional health care system like, treating swollen body part locally called *gofla*, colon cleaner, snakebite, liver swelling, spleen swelling/splenomegaly, fungal infections, and inflammation of skin (Anteneh and Bussa, 2014). The highly diversified bioactive compounds isolated from this *Aloe* species could be contributed for those diversified traditional values of *Aloe harlana*. The highly diversified bioactive compounds could create better and complex synergetic effects to be more effective in remedial application.

This narrow endemic *Aloe* species was found with morphological variability as spotted and non-spotted leaved in its natural habitat. This phytochemical analysis was done on the non-spotted specimen. However, highly spotted *Aloe harlana* with similar morphological characteristics to the non-spotted adult population were identified, which has not been phytochemically characterized in this study (Figure 17).



Figure 25. Non-spotted and spotted adult plants of *Aloe harlana* in the same locality of Harla (Photo courtesy: Anteneh Belayneh).

The taxonomy of *Aloe* at the species and infraspecific levels are under instability (Manning *et al.*, 2014; Cock, 2015). This can primarily be attributed to the fact that, *Aloe* species display

unusual patterns of variation among populations and species and inconsistent integration among them. Besides gross morphology, other characters have been employed by taxonomists to reveal the taxonomic confusion in the genus *Aloe* like, phytochemistry (Viljoen *et al.*, 2001) and molecular phylogeny (Grace *et al.*, 2013, Daru *et al.*, 2013; Manning *et al.*, 2014). Therefore, further phytochemical and molecular characterization of this morphologically different population of *Aloe harlana* should be done, which may leads to the taxonomic revision of this species.

4.1.3. *Aloe mcloughlinii* Chris.

Previous reports indicated that aloesin, dihydroisocoumaringlucoside, aloeresin D, aloin A/B, aloinoside A/B were isolated and characterized from *Aloe mcloughlinii* using HPLC method (Viljoen *et al.*, 2001). Additional seven compounds like, microdontin A and B, isorabichromone, aloeresin D, 5,3'-dihydroxy-6,7,4'-trimethoxyflavone(eupatorin), kaempferol-3-O-hexosyl-O-pentoside, and p-coumaric acid derivatives were isolated and some characterized in this study. The relatively higher concentration of commercial interest compound of aloin A (24%) makes this species compatible with the well known commercialized species of *Aloe ferox* with average reports of 28% aloin A (Chen *et al.*, 2012; Ombito *et al.*, 2015) and *Aloe barbadensis* Miller (syn. *Aloe vera*) average reports of 17-20% aloin A (Sánchez-Machado *et al.*, 2017). An in vivo studies have demonstrated the laxative, antioxidant, antimicrobial, and anti-cancer properties of anthraquinones and anthrones such as aloin (Park *et al.*, 2009; Chen *et al.*, 2012). Therefore, the newly isolated compounds from this species initiate in vivo and invitro studies of biological activities from its leaf exudate.

4.1.4. *Aloe megalacantha* Baker

The species level reports using HPLC method revealed that the leaf exudate of *Aloe megalacantha* contains dihydroisocoumaringlucoside, aloin A and B, aloinoside A and B, microdontin A and B, and aloe-emodin (Dagne and Alemu, 1991; Viljoen *et al.*, 2001). These reports did not indicate which sub species have been phytochemically analysed among the two sub species documented in Ethiopia. In this study, the phytochemical analysis from the leaf exudate of the two sub species of *Aloe megalacantha* known as *A. megalacantha* subsp. *megalacantha* and *A. megalacantha* subsp. *alticola* showed significance difference ($P > .05$).

Like previous reports on the phytochemical profile of *A. megalacantha* (Viljoen *et al.*, 2001) at the species level, in this study the sub species *A. megalacantha* subsp. *megalacantha* has microdantin A and B but not in the other sub species. Compounds such as isorabaichromone, kaempferol-3-O-hexosyl-O- pentoside, aloin B, microdantin A, and microdantin B are phenolic were isolated from *A. megalacantha* subsp. *megalacantha* that makes it different from the other sub species called *A. megalacantha* subsp. *alticola*.

It has been revealed that the sub species *A. megalacantha* subsp. *alticola* possesses 1-Hexanol-pentosylhexoside, Trihydroxy octadecenoic acid, 3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one (feralolide-3'-O- β -D-glucopyranoside), and aloeresin D. Therefore, dihydroisocoumarin, 3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one, 1-Hexanol-pentosylhexoside, trihydroxy octadecenoic acid, and aloeresin D are phenolic compounds that makes *Aloe megalacantha* subsp. *alticola* different from the other sub species of *A. megalacantha* subsp. *megalacantha*. Moreover, the absences of microdantin A and B in *Aloe megalacantha* subsp. *alticola*, which are the chemotaxonomic marker (aloin/aloinosie/microdantin group) for microdantin chemotype species that include *A. megalacantha* (Viljoen *et al.*, 2001), instigate taxonomic discussion on the current taxonomic position of *Aloe megalacantha* subsp. *alticola*.

This chemotaxonomic data supported that they could be of two different species rather than sub species of *Aloe megalacantha*. Therefore, the molecular analysis is strongly recommended to support this phytochemical data indicated more chemotaxonomic variation, which could lead them to be two different species of aloe rather than ranked as two sub species of *Aloe megalacantha*. Due to the complexities of the genus there are taxonomic problems encountered in the genus *Aloe* (Smith and Steyn, 2004; Grace *et al.*, 2013), and still taxonomic problems to be resolved are awaiting and new species are being discovered (Sebsebe and Gilbert, 2000), in which the chemotaxonomy will contribute.

In addition, the aloin A concentration 28% (*A. megalacantha* subsp. *megalacantha*) and 24% (*A. megalacantha* subsp. *alticola*) is comparable with the commercialized *Aloe* species (like, 28% in

A. ferox exudate, [Chen et al., 2012](#)). That could be contributed for local exploitation of its leaf exudate to process the laxative local product known as *sibrii* (Oromo language) and sold in the local markets from *A. megalacantha* subsp. *megalacantha* ([Belayneh and Bussa, 2014](#)).

4.1.5. *Aloe otallensis* Baker

The dried leaf exudate appearance and colour was much different from the common *Aloe* species dried exudate, which is not shiny and also ashy to grey coloured. Plicataloside (2,8-O,O-di (β -D-glucopyranosyl)-1,2,8-trihydroxy-3-methylnaphtalene) is the only compound reported so far from this species using [HPLC](#), preparative TLC and [NMR spectra](#) methods ([Viljoen et al, 1999](#); [Biniam et al., 2011](#)). However, additional new compound like, 1-O-methylplicataloside and 7-O-Methyl-2'-feruloylaloetin have been isolated and characterized from *A. otallensis* for the first time in this study. Following the traditional claim to treat malaria, the leaf exudate and plicataloside were evaluated for their *in vivo* antimalarial activity using a 4-day *Plasmodium berghei* suppressive test. The results indicated that exudate (300 mg/kg) and plicataloside (100 mg/kg) inhibited parasite growth by 60.7% and 40.7%, respectively ([Biniam et al., 2011](#)). The additional compounds isolated in this study could be in line with the better inhibition effect of leaf exudate than a single compound plicataloside. A plicataloside is a single marker compound in *A. otallensis* and *A. rugosifolia* having a taxonomic value for east African *Aloe* species, which has been grouped under the 20 *Aloe* species of plicataloside chemotype ([Viljoen et al., 1999](#)). Plicataloside concentration in *Aloe otallensis* (75%) is not significantly different ($P < .05$) from those plicataloside chemotype *Aloe* species like, *Aloe palmiformis* (82%), *A. plicatalis* (L.) Miller (82%), *A. tugenensis* L.E. Newton & Lavranos (79%), *A. pustuligemma* L.E. Newton (78%), *A. parvidens* (76%), and *A. schweinfurthii* Baker (59%) ([Viljoen, 1999](#)).

4.1.6. *Aloe pirottae* Berger

This endemic species of Ethiopia, which has not been analyzed for its phytochemical profile so far, showed the highest number of compounds from its leaf exudate. Among the 20 major peaks on the UHPLC-MS chromatogram, 15 compounds were isolated and characterized. The minor peaks at Rt 5.81, 8.40 (double peaks), 11.20, 11.38, and 13.20 were not analyzed for [M-H]⁻ ions *m/z* (Figure 16). Otherwise, the number of compounds that could be potentially isolated from the

UHPLC-MS chromatogram rose greater than the 15 identified and characterized compounds in this study. *Aloe pirottae*, a species endemic to Ethiopia, is valued as a remedy for malaria, tropical ulcer, gastro-intestinal parasites, gallstone, eye diseases and snake bite. The gel extracted from dried and ground plant material, called *sibri* (Oromo language), was acclaimed as a cleaner of the human colon (Belayneh *et al.*, 2012). The higher concentration of aloin should contribute for its effective laxative action. An in-vitro study on *Anopheles* mosquito revealed that 80% methanol leaf extract from *Aloe pirottae* was found as a promising mosquito larvicide (Bekele *et al.*, 2014). The synergetic effects of these compound complex could contribute for its remedial effect.

Unique compounds like, 1-Hexanol-pentosylhexoside (shared with *A. megalacantha* subsp. *alticola*), 6'-O-Coumaroyl aloesin (shared *A. trichosantha* subsp. *longiflora*), and Isorabichromone (shared with *A. megalacantha* subsp. *alticola* and *A. mcloughlinii*) were isolated from this species. The most important constituents isolated from commercial aloe (*A. arborescens*, *A. barbadensis*/*A. vera*, and *A. ferox*) like, aloin A and B, aloinoside A and B, microdantin A and B, aloesin, and aloeresin A (Chen *et al.*, 2012; Cock, 2015; El Sayed *et al.*, 2016; Salehi *et al.*, 2018) are all isolated from this species. The phtochemical profile of this specie was found much similar with the 36 *Aloe* species of microdantin chemotype groups.

4.1.7. *Aloe pubescens* Reynolds

The common anthrones like, aloin, homonataloin, and alinoside and the chromones like, aloesin and aloeresin has been reported from Ethiopian collection in leaf exudate of this species using HPLC method (Viljoen, 1999). Whereas, compounds like, 7-O-Methylaloesin, 7-Hydroxy-8-O-methylaloin B, and Aloe-emodin-8-O-glucoside (5-Hydroxyaloin-6'-acetate) are newly isolated and characterized from this species. Unlike the reports of Viljoen. (1999), homonataloin A and B were not isolated in this study. Compounds such as 7-O-Methylaloesin, 7-Hydroxy-8-O-methylaloin B, Aloe-emodin-8-O-glucoside (5-Hydroxyaloin-6'-acetate), and aloeresin G were isolated and characterized only from this endemic, stemless, and non-spotted *Aloe* species. About 54.5% of the compounds from the leaf exudate were unique to this *Aloe* species including unidentified peaks with [M-H]⁻ ions m/z 489.1763 and 491.1912. Therefore, beyond its unique flower morphology hairy inflorescence, its phytochemistry was found much different from the

Ethiopian *Aloe* species targeted in this study. This unique phytochemical profile could attract more research interest on its pharmacological activities.

4.1.8. *Aloe riva*e Baker

Among the nine compounds isolated from *A. riva*e, *deacetylitoraloin* is the unique compound isolated only from this species. Previous studies reported that dihydroisocoumarin glucoside, aloin A and B, aloinoside A and B, microdantin A and B, and aloe-emodin were isolated and characterized using TLC and HPLC methods (Dagne and Alemu, 1991; Viljoen *et al.*, 2001). Unlike these reports, dihydroisocoumarin glucoside and aloe-emodin, were not detected in this study, which could be due to the differences on collection sites and genetic variation of samples. The genetic materials and environmental variability are the key factors and major determinants of variation in the types and level of bioactive compounds in *Aloe* species (Amoo *et al.*, 2014; Cardarelli *et al.*, 2017). However, additional new compounds, which were not isolated before like, 3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one, *deacetylitoraloin*, and kaempferol-3-O-hexosyl-O-pentoside are isolated for *A. riva*e. Its unique phytochemistry could attract pharmacological studies, which has not been done so far.

4.1.9. *Aloe rugosifolia* Gilbert & Sebsebe

The non-shiny and ashy/grey coloured dried leaf exudate was found to have plicataloside as a dominant compound. In addition, additional compounds like, 1-O-methylplicataloside and 7-O-Methyl-2'-feruloylaloetin were isolated, which were not reported so far from this species. The concentration of plicataloside in *A. rugosifolia* (95%), was comparable with other monophyletic groups in which plicataloside was used as marker compound (Viljoen *et al.*, 2001). Therefore, the freeze-dried and powder leaf exudate could be used as a standard for the plicataloside compound without any further processing since it accounted for 95% of in a given mass of the dried leaf exudate.

4.1.10. *Aloe secundiflora* Engler

Aloenin, aloenin B, isobarbaloin (aloin B), barbaloin (aloin A), aloinside A, aloinside B, and aloesin derivative were reported from this species (Viljoen and VanWyk, 2000; Rebecca et al., 2003). The rest of the compounds, dihydroisocoumarin, aloesin, 6'-nalonylnatalion, aloeresin D, homonataloin A and B, isoaloeresin D, and *p*-coumaric acid derivatives are newly isolated phenolic compounds from *Aloe secundiflora*. A number of unidentified mixture of dimers were reported by Viljoen and van Wyk (2000), which are identified and characterized in this study. Unlike the previous reports, Aloin A was not detected in this study. Still, unresolved peaks at $[M-H]^-$ pseudomolecular ion (m/z) of 863.2368 with R_t 10.949 and 11.301 were not identified. This species was found with high diversity of bioactive compounds which could be promising *Aloe* species for further studies on its biological activities.

4.1.11. *Aloe tewoldei* Gilbert & Sebsebe

This study has isolated and characterized five new compounds such as, aloesin, 3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-O- β -D-glucopyranosyl-5'-hydroxyphenyl) methyl-2(1H)-benzopyran-1-one, 5,3'-Dihydroxy-6,7,4'-trimethoxyflavone (eupatorin), kaempferol-3-O-hexosyl-O-pentoside, and *p*-coumaric acid derivatives for this species. Other such as, dihydroisocoumarin glucoside, aloin A, aloin B, aloinside A, aloinside B, microdantin A, and microdantin B were isolated and characterized from this species using HPLC method (Viljoen et al., 2001). Due to the presence of aloin, aloinside, and microdantin in *A. tewoldei*, it has been grouped under the microdantin chemotype group of South and east African *Aloe* species chemotaxonomic group. The major peak at $[M-H]^-$ pseudomolecular ion (m/z) of 231.0669, which was not identified in this study was only detected from this species. In addition, it has one unique compounds of 5,3'-Dihydroxy-6,7,4'-trimethoxyflavone (eupatorin) (only shared with *A. mcloughlinii*). Such higher number of complex compounds could instigate further studies for its biological activities.

4.1.12. *Aloe trichosantha* subsp. *longiflora* Gilbert & Sebsebe

This is non-spotted endemic sub species of *Aloe trichosantha*. A report on the phytochemical profile from *Aloe trichosantha* indicated aloin A/B and aloin-6'-O-acetate A/B (Anwar et al.,

2014) at the species level, which did not differentiate the sub species type. The other report by Viljoen (1999) showed only aloin at the species level study, *Aloe trichosantha*. However, none of the aloins were isolated in this study at this sub species level. Therefore, the previous reports might be done on the other subspecies, *Aloe trichosantha* subsp. *trichosantha*, which could be potentially possess aloin A/B and aloin-6'-O-acetate A/B. The presence and absence of a marker compound known as aloin in these two sub species pose an issue of taxonomic discussion on these two sub species. The unique compounds isolated and characterized from this sub-species were 7-O-Methylaloeresin A, homonataloside A/B, aloeresin A, and [M-H]⁻ pseudomolecular ion (m/z) of 375.1092. So far, there was no any report on the phytochemistry at the sub species level for *Aloe trichosantha*, and to the knowledge of the authors this is the only report on phytochemical profile of an endemic sub-species *Aloe trichosantha* subsp. *longiflora*, which may pose taxonomic discussion of the two subspecies.

4.1.13. *Aloe yavellana* Reynolds

Most of the isolated compounds in this study such as Aloe-emodin, dihydroisocoumarin, Aloinoside A and B, Kaempferol-3-O-hexosyl-O- pentoside, and p-coumaric acid derivatives were not reported before from this narrowly endemic species. Aloin A/B and microdontin A/B were the only compounds reported so far from this species (Tibebu *et al.*, 2018). The leaf exudate and two major compounds isolated from it, namely, aloin A/B and microdontin A/B were assessed for their larvicidal activity against *Amblyomma variegatum* tick larvae using a larval packet test (LPT). Although both microdontin A/B and aloin A/B showed larvicidal activity, neither of the isolated compounds was as active as the leaf exudate suggesting that the compounds acted synergistically or other unidentified bioactive compounds with potent larvicidal activity may exist in the leaf exudate (Tibebu *et al.*, 2018). Likewise, this study has been revealed the presence of more bioactive compounds in the leaf exudate of *Aloe yavellana*. in addition, the presence of aloin, aloinoside, and microdontin could place *A. yavellana* in the microdontin chemotype chemotaxonomic group of south and east African Aloe species. However, the previous studies did not identified microdontins from this species (Dagne *et al.*, 2000; Viljoen *et al.*, 2001) so that, it was not categorized under microdontin chemotype chemotaxonomic group.

4.1.14. *Aloe retrospiciens* Reynolds & Bally and *Aloe ruspoliana* Baker

The physical parameters of leaf exudate of *Aloe retrospiciens* and *Aloe ruspoliana* were found to be much different from the common characteristics of *Aloe* leaf exudates. When the leaves of most species of *Aloe* are cut a more or less copious exudate appears, yellow at first but rapidly darkening to brown or in a few species dark red. Unlike this, the fresh leaf cutting of these two *Aloe* species do not show the common yellow coloured exudate release rather it appeared creamy white coloured exudate with unpleasant smell not common in *Aloe* species. The dried aloe exudate commercially known as aloe bitter has been produced by evaporating water using direct sunshine or boiling or by freeze drying to produce a yellow to brown hard and shining crystal/powder material (Steenkamp and Stewart, 2007). However, unlike this common characteristics of dried aloe exudate, the freeze dried exudates of these two species resulted in ashy coloured and non-powdered sticky solid. More importantly, unlike the powder of *Aloe* species exudates the sticky leaf exudates of *Aloe retrospiciens* and *Aloe ruspoliana* were found less soluble in distilled water. Extracts from *Aloe ruspoliana* gave positive test with Dragendorff's reagent indicating the presence of alkaloids with potential toxicity (Cock, 2015). However, this study could not identify any constituents from *Aloe retrospiciens* and *Aloe ruspoliana*. Either these techniques did not work for these samples or the collected samples were failed for unknown reasons. Hope these two species could attract further studies with different methods and design and molecular analysis are strongly recommended.

5. CONCLUSION

In this study, unique types of compounds were isolated and characterized, which were not reported so far from the Ethiopian *Aloe* species. For example, 1-O-methylplicataloside from *A. otallensis* and *A. rugosifolia*, deacetylitoraloin from *A. rivaie*, caffeoyl ester of aloesin from *A. harlana*, and aloenin B from *A. secundiflora* were unique to these species and not so far reported from Ethiopian *Aloe* species. More interestingly some *Aloe* species have compounds unique to a species only not shared with any one of the *Aloe* species targeted in this study. These could attract more research interest for their biological activities.

Most of the principal *Aloe* leaf exudate components such as, aloin A and B, aloinoside A and B, aloesin, microdontin A and B, homonataloin A and B, and aloe emodin were isolated and quantified from the majority of these targeted *Aloe* species of Ethiopia in which anthrones are the most important class of compounds found in most aloes. Derivatives of anthrone are used in pharmacy as laxative, which stimulate the motion of the colon and reduce water reabsorption. This result is very important, since in vivo and in vitro studies have demonstrated the laxative, antioxidant, antimicrobial, and anti-cancer properties of anthraquinones and anthrones. Among these anthrones, aloin was well known as a characteristic marker compound in most *Aloe* species including *A. arborescens*, *A. barbadensis*, and *A. ferox*, which are under large scale commercialization. This study showed similar pattern for aloin A and B in some *Aloe* species (like, *Aloe harlana*, *A. pirottae*, *A. tewoldei*, *A. megalacantha* subsp. *megalacantha*, *A. rivaie*, *A. mcloughlinii*, and *A. megalacantha* subsp. *alticola*) with relatively higher concentration in some species comparable to the concentrations reported to the well-known commercialized *Aloe* species. This makes these endemic *Aloe* species of Ethiopia better for aloin source compared to the well-known commercialized aloes. In addition, species like, *A. megalacantha* subsp. *megalacantha*, *A. pirottae*, *A. pubescens*, *A. secundiflora*, *A. trichosantha* subsp. *longiflora* and *A. mcloughlinii* could be considered as a good source of chromones such as aloeresins and aloesin. The higher concentration of plicataloside in *A. rugosifolia* (95%), nearly the dried exudate itself is a pure compound, which could be used as a standard without further processing. Moreover, the current taxonomic position of *Aloe megalacantha* subsp. *alticola* instigates taxonomic discussion due the significance difference of phytochemical profile of the two sub

species of *Aloe megalacantha*. In addition, lack of detecting any constituent from *Aloe retrospiciens* and *Aloe ruspoliana* in this study could attract further investigation for their phytochemical characterization.

In general, Ethiopian *Aloe* species have high diversity and unique phenolic compounds and characteristic features having a potential to be used for medicinal uses with further studies on their potential toxicity. In view of emerging trends of the *Aloe* products and enormous compounds isolated from Aloes, it was important to apply a comprehensive promotion of potential *Aloe* species to attract the emerging cosmetic and pharmaceutical industries in Ethiopia with their wide scale cultivation, in which the wild harvest is prohibited by CITES.

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RESEARCH ARTICLE

Acute oral toxicity test from leaf exudates of 17 Aloe species from East and South of the Great Rift Valley in Ethiopia

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Acute Oral Toxicity Test from Leaf Exudates of 17 *Aloe* Species from East and South of the Great Rift Valley in Ethiopia

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ABSTRACT

Ethiopian *Aloe* species were used for diverse traditional medicines but some species have been reported for potential toxicity. Therefore, oral acute toxicity test were done from leaf exudates of 17 *Aloe* species using Swiss albino mice (n=5/cage). A single oral dose of 300 and 2000mg/kg body weight at a rate of 1mL/100 gm administered and major toxicity symptoms were monitored; and relative body and organs weights were measured daily until day 14. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparison. Groups treated with exudates of *Aloe calidophila*, *A. trichosantha* subsp. *longiflora*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivaie*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei* and *A. yavellana* did not showed any signs of toxicity at both dose levels. These groups showed no significance difference ($P>.05$) in percentage body weight, relative organs weight and food and water consumption compared to control group and Median Lethal Oral Dose (LD_{50} -value) was estimated to be $>2000\text{mg/kg}$ body weight. Groups treated with *A. harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 showed overt signs of toxicity and significance difference ($P<.05$) of percentage change in body weight, body weight gain, relative liver weight, and food consumption compared to control group. *A. ruspoliana* and *A. retrospiciens* treatment groups showed severe level of toxicity signs and mortality of mice at both doses, LD_{50} -value was $>300\text{mg/kg}$ body weight. This acute oral toxicity test revealed the importance of safety concern while using *Aloe* species in traditional medicines.

Keywords: *Aloe* exudate, *A. pirottae*, *A. ruspoliana*, *A. retrospiciens*, toxic endemic *Aloe*, traditional medicine

1. INTRODUCTION

The genus *Aloe* L. had long history and given high rank as a multi-purpose herbal plant. It was renowned for its medicinal and cosmetic properties that have been exploited over millennia [1-5]. This is due to the presence of diverse and unique phytochemical compounds. The isolation, characterization, and efficacy evaluation of bioactive phytochemical compounds from the Ethiopian *Aloe* species were validated the existing traditional uses of aloes as well as the custodian of new compounds [6-10]. Many traditional healers and users were used *Aloe* species for medicinal and cosmetic purposes with the opinion that *Aloe* products are safe, however, this is not always the case with some *Aloe* species. There are few reports of toxic effects found in the literature reports. For example, in Kenya and Somalia it was reported that *A. ruspoliana* was used to kill hyenas by smearing meat with the leaf extract [11, 12]. *Aloe buettneri*, *A. lateritia*, *A. rabaiensis*, *A. secundiflora*, and *A. zebrina* have been documented as ingredients in arrow poisons throughout Africa [13]. These species have characteristic smell usually described as that of mice or rats associated with the presence of poisonous alkaloid, for which reason the East African *Aloe ballyi* is known as ‘the rat aloe’ [2].

Aloe species have potential toxicity, with side effects occurring at some dose levels when ingested or applied topically [14]. When the leaves of most species of *Aloe* are cut, a more or less copious exudate appears yellow at first but rapidly darkening to brown or in a few species dark red. This exudate contains phenolic compounds, which could be non-toxic in most *Aloe* species but some are extremely toxic, containing a hemlock like substance [15, 16]. Some *Aloe* species contain toxic alkaloids [17] and certain species, including *A. vera*, may cause adverse reactions [18]. There are published reports of human deaths resulted from the use of *Aloe* leaves [13, 19, 20], which could be resulted due to an over dose oral application of extracts for medicinal uses. There were also some expectedly poisonous *Aloe* species not studied so far, which were reported as poisonous by the local communities in Ethiopia [22]. The leaf juice of *Aloe* species was found from time to time in Africa arrow poison [21]. Mostly it was not known which species have been used except the following species are certain: *Aloe lateritia*, *A. rabaiensis* and *A. secundiflora* [13]. Somali hunters on the Wabi Shebelle in Ethiopia used the leaf of *Aloe* species in meat bait to poison animals [13]. That is why, beyond their wider uses, toxicological evaluation of *Aloe*

species is important for proper application of their products. Especially, the recent widespread exposure of the society to *Aloe* products in Ethiopia, it has been raised safety concerns and its potential toxic activities should be studied [22].

An increased popularity and utilization of *Aloe* species have been encouraged in-depth studies to investigate the adverse effects, conventional drug interactions, and the possible toxic and carcinogenic effects of *Aloe* preparations and their products [23]. Similarly, due to an increasing interest in the phytochemical and pharmacological potentials of *Aloe* species in Ethiopia, the need for toxicity tests of this valuable natural resource cannot be under-emphasized. The aim of toxicological evaluation of the targeted *Aloe* species is to assess their potential adverse effects. Therefore, this study has been focused on the acute oral toxicity test for the 17 prioritized *Aloe* species to generate basic scientific information, which could provide safety information for their existing diverse traditional medicinal applications, product development and promotion, utilization under cultivation, and foster wild conservation.

2. MATERIALS AND METHODS

2.1. Plant Materials

Fresh leaves of *Aloe* species from east and south of the Great Rift Valley floristic regions were collected from their corresponding natural habitats. Their taxonomic identification and authentication were done in the National Herbarium of College of Natural Sciences, Addis Ababa University (AAU). Voucher specimens were deposited at AAU and Haramaya University (HU) Herbaria. Exudates from the fresh leaves of the targeted *Aloe* species were collected by cutting the leaves transversally near the base and arranging them concentrically around the plastic plates to drain for 10-20 minutes. Then exudates of each species were poured in airtight plastic bottles, kept in field ice bag, and brought to laboratory for storage in the refrigerator until proceed to freeze-drying. The extracts were protected from any possible oxidation by being stored in airtight aluminium foil covered flasks. Then all exudates were freeze dried (Christ Alpha 1-2 LDplus, Germany) to be crystallized and powdered. The crystal/powder of each species were labelled and stored at 4⁰ C until used for acute oral toxicity test.

2.2. Experimental Animals

Experimental animals of outbreed female, nulliparous, and non-pregnant Swiss albino mice aged 6–8 weeks and weighing 23–30 gm were used for the experiment. Mice were obtained from experimental animals department of Ethiopian Public Health Institute (EPHI), Addis Ababa and interbreed at the animal house in central Laboratory of Haramaya University under proper care and management. Newly born female mice at the age of 21 days were separated in sexes and housed in polypropylene cages with stainless steel wire mesh cover ($n = 5/\text{cage}$). All groups were kept under 12 hours light and dark cycles at ambient room temperature (22-25⁰ C) of the toxicology laboratory of Haramaya University with free access to standard rodent pellet diet (from Addis Ababa Rodent feed preparation PLC) and water *ad libitum*. Before initiation of the experiment, animals were acclimatized to the laboratory conditions for one week. All procedures complied with the guide for care and use of Laboratory animals were in accordance with the guideline for the care and use of experimental animals stated in OECD 425 [24].

Experimentation Ethics Committee on animals' uses of the school of animals and range sciences of Haramaya University was approved the protocol used in this study.

2.3. Acute Oral Toxicity Test

Acute toxicity describes the adverse effects of a test substance resulted after administration of either from a single dose or multiple doses to a given concentration in a short period of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance [25]. In this study, acute oral toxicity test of the powdered leaf exudates from 17 *Aloe* species were evaluated using female Swiss albino mice (23–30 gm), according to the Organization of Economic Cooperation and Development (OECD) guideline 423 for testing of chemicals [26]. The total numbers of groups were 35 (17 *Aloe* species x 2 doses and one negative control group of distil water), with a total of 175 mice. Due to the more the numbers of treatment groups and number mice, the experiments were conducts in four rounds within two months of same season from February to March 2019. Initial body weight of each mouse were recorded after the fasting period of three hours and doses were calculated in reference to the body weight. The volume of the exudates powder solution given to the mouse was 1mL/100 gm.

Exudate powder of each *Aloe* species was dissolved in sterile distilled water and administered in a single loading dose of 300 and 2000 mg/kg body weight (each dose only once) at a rate of 1mL/100 gm orally by oral gavage (18 gauge). Whereas, the control group was receive sterile distilled water at the same rate of test substance. Food and water were provided an hour after dosing. Following oral administration of *Aloe* exudates, mice were observed individually and periodically during the first 24 hours (0.5, 1, 2, 4, and 12 hrs) with special attention given to the first 4-6 hours, and daily thereafter for a total of 14 days [26]. Then toxic symptoms and behavioural changes such as, piloerection, sedation or sleep, hyperactivity, restlessness, writhing, tremor, convulsion, lethargy, salivation, lacrimation, loss of appetite, diarrhoea, and mortality were monitored and recorded accordingly [27, 28]. The median lethal dose (LD₅₀ oral [p.o.]) was estimated based on mortality recorded within 24 hrs and the body weights of the mice were measured on days 7 (week 1) and 14 (week 2) following OECD guideline 423 [26]. Then two

week after, all animals were euthanized by CO₂ inhalation at the end of the experiment, and major organs (liver, kidney, heart, and spleen) were removed, cleaned, observed for gross pathology, and measured.

2.4. Data Analysis

The data were organized and cleaned in Excel data sheet, office 2016, to be suitable for both qualitative and quantitative analysis. R software version 3.3.3. was used for data analysis. Data were presented as mean $\pm$ SEM with 95% confidence interval for mean percentage body weight change, final body weight gain, mean relative organs' weight change in relation to absolute final body weight and food and water consumption. The relative organ weight was calculated as absolute organ weight (gm)/body weight of mice on the sacrifice day (gm) $\times$ 100. The percentage of body weight change was calculated as body weight at the end of each week minus initial body weight divided by initial body weight multiplied by 100. Then one-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons of the mean differences and responses of the two doses of *Aloe* exudates. The mean difference between groups with respect to impact of doses under investigation was significant at P value of less than 0.05 compared to the negative control.

3. RESULTS

3.1. General Signs and Behavioral Analysis of Oral Acute Toxicity Test

Many *Aloe* species were used for traditional medicinal and cosmetic purposes by most of the traditional healers and local users in Ethiopia with the opinion that *Aloe* products are safe; however, this is not always the case with some *Aloe* species [22]. That is why this study has been necessitated the safety of traditionally used *Aloe* species for medicinal purposes so that acute oral toxicity has been investigated to produce baseline information on the safety of these targeted *Aloe* species. Exudates from 17 *Aloe* species (*Aloe calidophila*, *A. harlana*, *A. mcloughlinii*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pirottae*, *A. pubescens*, *Aloe retrospiciens*, *A. rivaie*, *A. rugosifolia*, *A. ruspoliana*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora*, *A. yavellana*, and unidentified species/AHU53) were analyzed for any signs of toxicity and behavioural changes

A single loading oral dose of leaf exudate from 11 *Aloe* species (*A. calidophila*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivaie*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora* and *A. yavellana*) did not induced any overt signs of toxicity and behavioural changes at the starting dose of 300 mg/kg during the 14 days of observation. However, mice administered with exudates from *A. calidophila*; *A. megalacantha* subsp. *alticola*; *A. megalacantha* subsp. *megalacantha*, *A. pubescens*, *A. rivaie*, *A. secundiflora*, *A. tewoldei*, and *A. yavellana* showed passive behavioural change at the next higher dose of 2000 mg/kg such as, piloerection, restlessness, and tremor in the first 24 hrs of the assay yet all of which disappeared then after. There was no mortality recorded from these 11 *Aloe* species at 300 and 2000 mg/kg body weight. In addition, most groups, except groups treated with *A. otallensis*, *A. retrospiciens*, *A. rugosifolia*, *A. ruspoliana*, and *A. trichosantha* subsp. *longiflora* were exhibited diarrhoea 2-6 hrs post-administration of the exudates at 300 and 2000 mg/kg doses. Different degrees of diarrhoea were noticed at the same dose level among different treatment groups. Among the diarrheic groups, those treated with exudates from *A. harlana*, *A. megalacantha* subsp. *megalacantha*, and *A. pirottae* were induced higher degree of diarrhoea at both dose levels.

Groups treated with exudates from *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and unidentified/AHU53 were induced slight behavioural changes at lower dose of 300 mg/kg body weight such as, piloerection and restlessness in few hours of post-administration, and disappeared soon after. The groups of mice treated with same four species above at higher dose of 2000 mg/kg body weight showed passive behavioural and physiological changes such as, piloerection, restlessness, tremors, and loss of appetite 2 to 4 hrs after oral administration up to 24 hrs of the assay with improvement day after day, which disappeared after 72 hrs.

Two near endemic *Aloe* species, *Aloe retrospiciens* and *A. ruspoliana* (Figure 1), showed overt signs of toxicity at both dose levels of 300 and 2000 mg/kg body weight. The leaf exudates of these *Aloe* species were found much different from the common natural characteristics of *Aloe* leaf exudates. The fresh leaf cutting of most *Aloe* species were known to release a more or less copious exudate appears yellow at first but rapidly darkening to brown or in a few species dark red. Unlike this, the fresh leaf cutting of these two *Aloe* species did not showed these common exudate colours rather appeared creamy white coloured exudate with unpleasant smell, which was not common in *Aloe* species. Freeze-dried exudates of these two species resulted in ashy coloured and sticky solid rather than the commonly known powder of *Aloe* species. More importantly, unlike the powder of most *Aloe* species exudates, the leaf exudates of *Aloe retrospiciens* and *A. ruspoliana* were found less soluble in distilled water.



Fig 1. *Aloe retrospiciens* (left) and *A. ruspoliana* (right) in their respective natural habitat(Photo courtesy: Anteneh Belayneh).

A group treated with leaf exudate of *A. ruspoliana* at the dose of 300 mg/kg showed overt behavioural changes but no mortality recorded. Whereas, adverse behavioural changes were noticed at the next higher dose of 2000 mg/kg body weight such as slow response to external stimuli, reduction of mobility and aggression, convulsion, tremor and writhing and one mortality per group of n=5 (Figure 2). The leaf exudate of *Aloe retrospiciens* has been showed over signs of toxicity and behavioural changes at both dose levels as well as resulted in mortality of one mouse at the dose of 300 mg/kg and three mice (50%) at the dose of 2000 mg/kg (n=5 in each treatment group) after single dose oral administration.

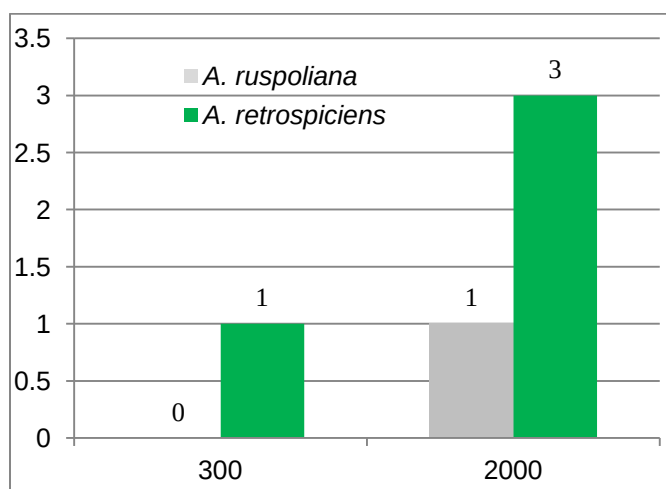


Fig 2. Number of deaths (y-axis) from exudate of *Aloe retrospiciens* and *A. ruspoliana* treatment groups at the doses of 300 and 2000 mg/kg (x-axis).

3.2. Absolute and Relative Body and Organs Weight Changes

3.2.1. Percentage Change in Mean Absolute Body Weight

The percentage change in mean absolute body weight at week 1 and week 2 as well as body weight gain at the fourteenth day were found lower for all test groups compared to the negative control group. However, most *Aloe* species (except, *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and unidentified AHU53) showed no significant difference ($P > .05$) in both weeks compared to the control group (Table 1). Groups treated with exudates from *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and AHU53 exhibited significant difference ($P < .05$) in percentage change in mean absolute body weight at week 1 and week 2 at both dose levels of 300 and 2000 mg/kg body

weight compared to the control group (Table 1), except *A. pirottae* (P=.471) on week 1 at 300 mg/kg dose.

Table 1. Percentage change in mean body weight with standard error of mean (Mean±SEM) at week 1 and week 2 and level of significance difference compared to the negative control group

Code of TG	Treatment group	Exudate dose mg/kg	Mean body wt at day 0 (gm)	Percentage change in mean body wt			
				Week 1 %	P value	Week 2 %	P value
.0	Negative Control	Distil water	25.3±.37	4.35±1.13		11.73±2.02	
1.1	<i>Aloe calidophila</i>	300	25.8±.39	1.94±0.18	1.000	8.16±1.9	1.000
1.2		2000	24.7±.7	2.47±0.81	1.000	6.94±1.3	.999
2.1	<i>Aloe harlana</i>	300	25.7±.79	-4.82±2.1	.032*	-3.07±2.3	.000***
2.2		2000	25.7±.88	-10.09±1.8	.000***	-7.3±1.9	.000***
3.1	<i>Aloe mcloughlinii</i>	300	26.4±1.1	-5.75±1.5	.008**	-8.65±5.01	.000***
3.2		2000	25.4±.69	-5.79±1.13	.007**	-12.17±2.1	.000***
4.1	<i>A. megalacantha</i> subsp. <i>alticola</i>	300	27.0±.79	3.73±.88	1.000	9.73±1.5	1.000
4.2		2000	24.6±.53	1.68±1.17	1.000	5.29±2.1	.925
5.1	<i>A. megalacantha</i> subsp. <i>megalacantha</i>	300	25.7±.39	3.24±1.0	1.000	8.44±1.6	1.000
5.2		2000	24.9±.4	-1.23±.5	.827	4.07±2.1	.687
6.1	<i>Aloe otallensis</i>	300	26.2±.6	3.62±.5	1.000	9.39±.9	1.000
6.2		2000	24.7±.62	2.86±.53	1.000	5.7±2.6	.963
7.1	<i>Aloe pirottae</i>	300	25.9±.49	-2.35±2.3	.471	-.07±1.5	.025*
7.2		2000	26.1±.57	-3.07±.76	.257	-4.56±.94	.000***
8.1	<i>Aloe pubescens</i>	300	26.9±.64	3.77±.80	1.000	9.3±1.4	1.000
8.2		2000	25.8±.8	2.26±1.15	1.000	7.69±1.7	1.000
9.1	<i>Aloe rivae</i>	300	25.8±.8	1.88±.57	1.000	5.75±.73	.967
9.2		2000	24.8±.64	.68±2.89	.999	4.98±1.5	.882
10.1	<i>Aloe rugosifolia</i>	300	25.4±.47	4.22±.76	1.000	9.4±2.5	1.000
10.2		2000	25.0±.55	3.24±.54	1.000	6.12±2.3	.985
11.1	<i>Aloe secundiflora</i>	300	26.7±.88	-2.08±.73	.561	3.62±.98	.569
11.2		2000	26.2±.86	-1.47±.69	.760	3.57±2.5	.554
12.1	<i>Aloe tewoldei</i>	300	25.5±.79	1.61±2.16	1.000	6.09±2.3	.984
12.2		2000	25.2±.58	1.05±3.44	1.000	4.91±2.8	.870
13.1	<i>Aloe trichosantha</i>	300	26.7±.88	.92±1.6	1.000	3.74±1.9	.601
13.2	subsp. <i>longiflora</i>	2000	25.2±.97	2.19±3.75	1.000	4.49±1.3	.789
14.1	<i>Aloe yavellana</i>	300	25.5±.91	2.40±1.1	1.000	7.05±.76	.999
14.2		2000	25.2±.72	1.42±1.1	1.000	4.63±.78	.818
15.1	Unidentified (AHU53)	300	25.5±.87	-4.99±1.16	.025*	-7.84±2.8	.000***
15.2		2000	26.2±1.03	-7.996±2.9	.000***	-11.86±1.7	.000***
Total	Total	N=186	25.7±.13	-0.003±.4		3.06±.62	

TG = treatment group; N= total number of mice; P value = Mean difference of test groups is significant at the 0.05 level compared to the negative control; level of significance difference from lower to higher = *p < 0.05; **p < 0.005; ***p < 0.0005.

The mean body weight gain on the fourteenth day of the test groups treated with exudates of *Aloe calidophila*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivae*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha*

subsp. *longiflora* and *A. yavellana* were not significantly different ($P>.05$) from the control group at both dose levels. However, it was significantly different ($P<.05$) for groups treated with exudates of *Aloe harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 at both dose levels, with negative values of -3.14 and -0.04, compared to the negative control group (Table 2). Especially, the body weight gain was dropped more significantly for unidentified/AHU53, -3.14±.53 ($P=.0001$), followed by *A. mcloughlinii* with -3.1±.53 ($P=.0001$) at 2000 mg/kg dose, which are much significantly different ($***p <.0005$) from the control group (Table 2).

Table 2. The mean absolute body weight gain with standard error of mean (Mean±SEM) and level of significance difference of treated groups compared to the negative control group

Code of TG	Treatment group	Exudate dose mg/kg	Initial body wt at day 0	final body wt at day 14	Body wt gain	P value for body wt gain
.0	Negative Control	Distil water	25.3±.37	28.3±0.59	2.96±.51	
1.1	<i>Aloe calidophila</i>	300	25.8±.39	27.9±0.61	2.1±.49	1.00
1.2		2000	24.7±.7	26.4±0.69	1.7±.3	.998
2.1	<i>Aloe harlana</i>	300	25.7±.79	24.9±1.14	-0.76±.59	.001**
2.2		2000	25.7±.88	23.8±0.85	-1.9±.53	.0004***
3.1	<i>Aloe mcloughlinii</i>	300	26.4±1.1	24.1±1055	-2.3±1.35	.0002***
3.2		2000	25.4±.69	22.3±0.73	-3.1±.53	.0001***
4.1	<i>A. megalacantha</i> subsp.	300	27.0±.79	29.6±0.73	2.6±.37	1.00
4.2	<i>alticola</i>	2000	24.6±.53	25.9±0.75	1.3±.51	.922
5.1	<i>A. megalacantha</i> subsp.	300	25.7±.39	27.8±0.12	2.14±.39	1.000
5.2	<i>megalacantha</i>	2000	24.9±.4	25.9±0.53	1.0±.52	.696
6.1	<i>Aloe otallensis</i>	300	26.2±.6	28.6±0.48	2.82±.2	1.00
6.2		2000	24.7±.62	26.1±0.82	2.4±.62	1.00
7.1	<i>Aloe pirottae</i>	300	25.9±.49	25.6±0.33	-0.04±.39	.029*
7.2		2000	26.1±.57	23.6±0.51	-1.2±.25	.006**
8.1	<i>Aloe pubescens</i>	300	26.9±.64	29.4±0.68	2.5±.35	1.00
8.2		2000	25.8±.8	27.8±1.08	2.0±.47	1.00
9.1	<i>Aloe rivae</i>	300	25.8±.8	27.3±0.99	1.5±.22	.982
9.2		2000	24.8±.64	26.0±0.35	1.2±.33	.865
10.1	<i>Aloe rugosifolia</i>	300	25.4±.47	27.8±0.34	2.64±.62	1.00
10.2		2000	25.0±.55	26.5±0.5	2.5±.55	1.00
11.1	<i>Aloe secundiflora</i>	300	26.7±.88	27.6±0.73	0.94±.25	.635
11.2		2000	26.2±.86	27.1±0.87	0.9±.64	.593
12.1	<i>Aloe tewoldei</i>	300	25.5±.79	27.1±1.1	1.56±.61	.990
12.2		2000	25.2±.58	26.4±0.62	1.2±.72	.865
13.1	<i>Aloe trichosantha</i>	300	26.7±.88	27.6±0.53	0.94±.49	.635
13.2	subsp. <i>longiflora</i>	2000	25.2±.97	26.3±0.82	1.1±.29	.788
14.1	<i>Aloe yavellana</i>	300	25.5±.91	27.3±1.01	1.8±.2	.999
14.2		2000	25.2±.72	26.4±0.71	1.16±.19	.836
15.1	Unidentified (AHU53)	300	25.5±.87	23.5±1.05	-2.0±.72	.0002***
15.2		2000	26.2±1.03	23.1±0.8	-3.14±.53	.0001***

3.2.2. Relative Organs Weight Changes

Percentage of four major organs (liver, kidney, heart and spleen) weight in relation to body weight or relative organs' weight (g%) for each mouse at day 15 of the experiment, which were induced exudates from 15 *Aloe* species, were measured. Then the mean of each test group was compared with the negative control group to see if significant difference in relative mean organs weight (g%). None of the treatment groups showed significance difference ($P>.05$) in percentage weight of kidney, heart and spleen compared to the control group at 300 and 2000 mg/kg body weight dose levels (Table 3). However, percentage of liver weight in relation to the final body weight has showed significant difference ($P<.05$) for groups treated with exudates of *Aloe harlana* and *A. pirottae* at 2000 mg/kg body weight. While, groups treated with exudates of *Aloe mcloughlinii* and Unidentified/AHU53) were significantly different ($P<.05$) at both dose levels of 300 and 2000 mg/kg body weight (Table 3).

Table 3. Percentage of organs' (liver, kidney, heart and spleen) weight in relation to body weight or relative organs' weight (g%) at day 15 of the experiment

Code of TG	Exudate dose mg/kg	Percentage of liver	P value	Percentage of kidney	P value	Percentage of heart	P value	Percentage of spleen	P value
.0	Distil water	5.19±.10		1.35±.026		.57±.02		.45±.01	
1.1	300	5.25±.11	1.00	1.31±.024	1.00	.57±.02	1.00	.395±.02	.993
1.2	2000	5.77±.11	.628	1.23±.02	.720	.58±.01	1.00	.39±.01	.987
2.1	300	5.88±.24	.592	1.24±.046	.827	.61±.02	1.00	.38±.01	.787
2.2	2000	6.15±.21	.036*	1.24±.05	.884	.63±.01	.998	.38±.02	.827
3.1	300	6.19±.40	.016**	1.24±.04	.842	.61±.02	1.00	.42±.04	1.00
3.2	2000	6.53±.19	.001***	1.22±.02	.586	.60±.04	1.00	.37±.01	.547
4.1	300	4.96±.1	1.0	1.32±.05	1.00	.54±.01	1.00	.41±.01	1.00
4.2	2000	5.34±.11	1.00	1.315±.02	1.00	.57±.02	1.00	.37±.01	.626
5.1	300	5.27±.03	1.00	1.30±.027	1.00	.55±.02	1.00	.39±.03	.959
5.2	2000	5.24±.11	1.00	1.27±.013	.996	.53±.02	1.00	.38±.02	.805
6.1	300	5.15±.1	1.00	1.35±.01	1.00	.55±.03	1.00	.43±.01	1.00
6.2	2000	5.63±.19	.894	1.25±.04	.971	.54±.02	1.00	.40±.02	.998
7.1	300	5.68±.08	.871	1.18±.045	.093	.58±.03	1.00	.39±.02	.940
7.2	2000	6.09±.13	.041*	1.197±.02	.221	.62±.01	1.00	.37±.01	.603
8.1	300	5.02±.12	1.00	1.31±.027	1.00	.55±.01	1.00	.40±.01	.999
8.2	2000	5.29±.2	1.00	1.38±.033	1.00	.58±.01	1.00	.42±.01	1.00
9.1	300	5.37±.18	1.00	1.36±.02	1.00	.59±.01	1.00	.43±.03	1.00
9.2	2000	5.60±.07	.897	1.28±.03	1.00	.62±.03	1.00	.42±.01	1.00
10.1	300	5.29±.08	1.00	1.33±.02	1.00	.57±.02	1.00	.43±.01	1.00
10.2	2000	5.49±.09	1.00	1.28±.027	1.00	.57±.03	1.00	.36±.02	.239
11.1	300	5.3±.13	1.00	1.29±.04	1.00	.57±.04	1.00	.38±.03	.778
11.2	2000	5.22±.15	1.00	1.21±.03	.452	.58±.03	1.00	.35±.02	.112
12.1	300	5.42±.19	1.00	1.29±.03	1.00	.57±.03	1.00	.36±.03	.267
12.2	2000	5.50±.13	1.00	1.31±.04	1.00	.59±.04	1.00	.37±.02	.632

13.1	300	5.29±.13	1.00	1.31±.013	1.00	.59±.02	1.00	.42±.01	1.00
13.2	2000	5.44±.15	.996	1.25±.03	.955	.55±.03	1.00	.36±.02	.436
14.1	300	5.00±.16	1.00	1.32±.02	1.00	.54±.04	1.00	.37±.01	.491
14.2	2000	5.22±.12	1.00	1.25±.02	.962	.56±.02	1.00	.42±.03	1.00
15.1	300	6.25±.29	.007**	1.26±.04	.983	.61±.03	1.00	.40±.03	.999
15.2	2000	6.34±.24	.002**	1.25±.05	.972	.64±.02	.995	.37±.02	.697
Total	N=186	5.51±.04		1.28±.01		.58±.004		.39±.003	

The gross pathology examination on liver and kidney of all treatment groups for potential signs of hepatotoxicity and nephrotoxicity showed no overt abnormal signs in colour, size, shape, and texture compared to the control group.

3.3. Food and water consumptions

The food consumption of groups treated with exudates from *A. calidophila*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivae*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora* and *A. yavellana* were not significantly different ($P>.05$) compared to the control group measured throughout the study period (Table 4). However, food consumption had showed significant difference ($P<.05$) for groups treated with exudates of *Aloe harlana* and *A. pirottae* at 2000 mg/kg body weight while groups treated with exudates of *Aloe mcloughlinii* and unidentified/AHU53 were significantly different ($P<.05$) at both dose levels of 300 and 2000 mg/kg body weight (Table 4). The water intake of all treatment groups were not significantly different ($P>.05$) at both dose levels compared to the negative control group.

Table 4. Food and water consumption of mice treated with *Aloe* exudates

Code of TG	Treatment group	Exudate dose mg/kg	Food consumption in gm per week (Mean±SEM)			Water intake in ml per week (Mean±SEM)		
			Week 1	Week 2	P value	Week 1	Week 2	P value
.0	Negative control	Distil water	18.7±.008	20.4±.008		26.6±0.01	28.2±0.01	
1.1	<i>Aloe calidophila</i>	300	20.4±.008	23.2±.008	.698	29.6±.00	30.6±.00	1.0
1.2		2000	16.6±.004	18.6±.004	.671	28.5±.01	31.5±.01	1.0
2.1	<i>Aloe harlana</i>	300	14.5±.008	16.5±.008	.262	26.5±.01	28.5±.01	1.0
2.2		2000	12.6±.009	13.6±.009	.021*	32.5±.01	30.5±.01	.686
3.1	<i>Aloe mcloughlinii</i>	300	12.4±.009	13.4±.009	.024*	28.5±.01	28.5±.01	1.0
3.2		2000	10.5±.007	11.5±.007	.001**	32.3±.01	28.3±.01	.946
4.1	<i>A. megalacantha</i>	300	14.4±.007	19.8±.007	1.00	26.6±.01	28.6±.01	1.0
4.2	subsp. <i>alticola</i>	2000	15.6±.016	18.5±.016	.871	30.5±.01	29.5±.01	1.0
5.1	<i>A. megalacantha</i>	300	14.2±.007	17.8±.007	.678	27.5±.01	30.5±.01	1.0
5.2	subsp. <i>megalacantha</i>	2000	16.4±.008	18.2±.008	.692	31.4±.01	28.4±.01	.948
6.1	<i>Aloe otallensis</i>	300	19.4±.005	20.4±.005	1.00	26.6±.01	28.6±.01	1.0
6.2		2000	18.0±.004	19.4±.004	1.00	29.4±.01	30.4±.01	.982
7.1	<i>Aloe pirottae</i>	300	14.4±.005	16.4±.005	.668	27.5±.01	26.5±.01	1.0
7.2		2000	12.5±.005	14.5±.005	.024*	26.5±.01	26.5±.01	1.0
8.1	<i>Aloe pubescens</i>	300	18.4±.006	20.7±.006	1.00	30.6±.01	28.6±.01	1.0
8.2		2000	17.6±.004	19.6±.004	1.00	31.6±.01	30.6±.01	1.0
9.1	<i>Aloe rivae</i>	300	20.4±.012	21.4±.012	1.00	26.6±.01	28.6±.01	1.0
9.2		2000	18.6±.007	18.4±.007	.985	29.6±.01	30.6±.01	1.0
10.1	<i>Aloe rugosifolia</i>	300	21.4±.005	22.4±.005	1.00	28.6±.00	26.6±.00	1.0
10.2		2000	18.5±.005	18.5±.005	.982	26.5±.01	27.5±.01	1.0
11.1	<i>Aloe secundiflora</i>	300	17.6±.005	18.4±.005	.719	28.6±.02	30.6±.02	1.0
11.2		2000	15.2±.01	18.4±.01	.568	32.6±.01	28.6±.01	1.0
12.1	<i>Aloe tewoldei</i>	300	15.6±.005	18.6±.005	.819	29.5±.01	28.5±.01	1.0
12.2		2000	13.5±.008	16.5±.008	.668	31.6±.01	30.6±.01	1.0
13.1	<i>Aloe trichosantha</i>	300	14.6±.01	17.6±.01	.898	26.6±.01	28.6±.01	1.0
13.2	subsp. <i>longiflora</i>	2000	13.4±.006	17.4±.006	.885	31.5±.01	28.5±.01	1.0
14.1	<i>Aloe yavellana</i>	300	18.8±.013	20.3±.013	1.00	26.5±.01	29.5±.01	1.0
14.2		2000	16.7±.015	18.5±.015	.855	31.5±.01	32.5±.01	1.0
15.1	Unidentified	300	12.4±.009	13.4±.009	.022*	26.4±.00	29.4±.00	.995
15.2	(AHU53)	2000	10.5±.005	12.5±.005	.001***	25.5±.00	26.5±.00	.895

4. DISCUSSION

4.1. General Signs and Behavioral Analysis

Despite the growing popularity and presumed safety of herbal medicines, adverse effects have become a major safety issue for natural products [29]. Consequently, in response to public health concerns, it is important to validate the safety of traditional herbal medicine before their use [30]. That is why; 17 prioritized *Aloe* species were tested for acute oral toxicity at 300 and 2000 mg/kg body weight in a single oral dosing. Acute toxicity test method with a starting dose of 300 mg/kg body weight was primarily used in a situation where there has no information indicating the test material is likely to be toxic [26]. There were no any toxicity experiments reported on the 17 *Aloe* species targeted in this study. Therefore, the starting dose of 300 mg/kg body weight has been used and revealed that no any signs of toxicity and behavioural changes in groups treated with exudates of *Aloe calidophila*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivae*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora* and *A. yavellana*. So that the next higher dose, 2000 mg/kg of these *Aloe* species were given to the test groups and induced passive behaviour in the first 24 hrs of the assay and all of which disappeared after 24 hrs indicating the non-toxic effect of these exudates. No mortality was observed in these treated groups, i.e., at 300 and 2000 mg/kg during the study period of 14 days. Therefore, the LD50 of these exudates could be possibly greater than 2000 mg/kg. The exudates of these *Aloe* species might be considered relatively safe on acute exposure. Because, the observation that oral administration of exudates from 11 *Aloe* species mentioned above did not caused any mortality or altered any behavioural and physiological state of mice in the acute toxicity tests indicating not harmful at the level tested and potentially non-toxic. This support the reason why most of these *Aloe* species were used widely as a therapeutic remedy both topically and orally by the local communities [22, 31-33]. Their toxicity characteristics were more similar with aqueous extract of *A. ferox* [34], which is popular in therapeutic remedy both topically and orally as well as in consumer products [24].

The groups treated with exudates from *Aloe calidophila*, *A. harlana*, *A. mcloughlinii*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. pirottae*, *A. pubescens*, *A. rivae*, *A. secundiflora*, *A. tewoldei*, *A. yavellana*, and unidentified/AHU53 were induced

different levels of diarrhoea at both dose levels. This could be due to the presence of the most common *Aloe* compound in these *Aloe* species known as aloin, which have laxative effect [35, 36]. That is why some of these *Aloe* species were used by the local communities as a colon cleaner [22]. The latex extracted from leaf and ground plant material of *A. pirottae*, called *sibri* (Oromo language), was acclaimed as a cleaner of the human colon [32]. The laxative local product known as *sibrii* (Oromo language) was processed and sold in the local markets from *A. megalacantha* subsp. *megalacantha* [33]. The different degree of diarrhoea among the different groups treated with different exudates at same dose level could be due to the difference in aloin concentration (with equal dry weight of aloe exudates) among the *Aloe* species. The amount of aloin is extremely variable among different species and highly depends on the growing conditions [37, 38]. The groups treated with exudates of *A. otallensis*, *A. rugosifolia* and *A. trichosantha* subsp. *longiflora* did not induced diarrhoea, which could indicate the absence of aloin. There were no any phytochemical studies reported aloin from *A. otallensis* and *A. rugosifolia* [39, 40]. Similarly, an endemic species of *A. trichosantha* subsp. *longiflora* may not have aloin among its secondary metabolites.

The results demonstrated that four *Aloe* species i.e. *Aloe harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 were induced overt response to external stimuli for 24 hrs while administered with 300 mg/kg body weight. Whereas, groups administered 2000 mg/kg body weight dose were induced further behavioural changes and became more diarrheic, which has been affected the body weight gain at the 14th day. That is why exudates of these *Aloe* species were considered as having potential toxic effects at the higher dose levels. The widespread human exposure to *Aloe vera* products have been raised safety concerns and its potential toxic activities were well studied [23]. Similarly, as these four species are used in the traditional health care system for treatment of diverse ailments, their safety concern need to be given attention. For example, the narrowly endemic *Aloe harlana* was reported to be used for a number of ailments like, treating swollen body part locally called *gofla*, colon cleaner, snakebite, liver swelling, spleen swelling/splenomegaly, fungal infections, and inflammation of skin [33]. Its leaf exudate was dried, crystallized and powdered for the preparation of a popular traditional colon cleaner locally known as *sibri* (Oromo language), sold on local market places. Therefore, *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and unidentified/AHU53 require detailed analysis including sub

acute and chronic toxicity tests. The results from this acute oral toxicity test showed that the exudate concentrations above 2000 mg/kg body weight could result toxicity in any form of oral application from these four *Aloe* species. That means, the LD₅₀ lethal dose that causes death in 50% of test animals was higher than 2000mg/kg body weight for exudates of *Aloe harlana*, *A. mcloughlinii*, *A. pirottae*, and Unidentified/AHU53 in accordance with OECD [26]. It has been reported that some *Aloe* species have potential toxicity, with side effects occurring at some dose levels when taken orally or applied topically [14].

Two endemic *Aloe* species i.e. *Aloe retrospiciens* and *A. ruspoliana* were resulted in mortality of 1-3 mice at 300 mg/kg and 2000 mg/kg body weight few hours after single dose oral administration of the exudate indicated the higher toxic effect of edudates from these *Aloe* specie. An increase in mortality is proportional to increase in concentration, which provided linearity in the dose-effect relationship of any extract and determination of the LD₅₀ value [35, 41], which has been seen in these treatment groups too. The LD₅₀ lethal dose that causes death in 50% of test animals was higher than 300mg/kg body weight for exudates of *Aloe retrospiciens* while the LD₅₀ lethal was higher than 2000mg/kg body weight for exudates of *A. ruspoliana*. In addition, *A. ruspoliana* leaf exudate could be classified under GH4 category above the concentration of 300 mg/kg body weight of the leaf exudate. According to OECD criteria, the occurrence of toxicity sign in more than one animal and/or $\leq$ one death classified the test substance in the GH4 category, indicating a warning [26]. Though there is no or single report on the phytochemical constituents of these two species so far, some alkaloids and/or potentially toxic compounds could be expected to pose the toxicity. Alkaloids, especially the ones belonging to the hemlock class, have been reported in some toxic species such as *A. sabaena* [42, 43]. Quite a few number of *Aloe* species were reported as poisonous, because of the presence of the hemlock alkaloid γ -coniciene (oral LD₅₀ = 12 mg/kg for mice) in exudate of leaves [15]. In Kenya and Somalia, it was reported that *A. ruspoliana* is used to kill hyenas by smearing meat with the leaf extract [12]. A report indicated a positive test with Dragendoff's reagent indicating the presence of alkaloids from the leaf extract of *A. ruspoliana* [44]. To the best of our knowledge, this is the first *Aloe* toxicity experimental report from Ethiopian *Aloe* species, which could attract detail toxicological and phytochemical studies for public safety reason due to the current increasing public interest of *Aloe* products in Ethiopian.

4.2. Absolute and Relative Body and Organs Weight Changes

4.2.1. Percentage Change in Mean Absolute Body Weight

The percentage change in mean absolute body weight of most test groups were less compared to the control group (not significantly different, $P > .05$). This could be attributed to the impact of different degree of diarrhoea occurred in most test groups in few hours after oral administration of the exudates due to laxative effect of aloin compound rather than toxicity. The percentage change in mean absolute body weight of *A. otallensis* and *A. rugosifolia* treatment groups showed more related values, at both dose levels, with the negative control group compared to the other treatment groups. The phytochemical analysis of *A. otallensis* and *A. rugosifolia* indicated that no aloin in their phytochemical constituents [36]. That is why, the groups treated with these *Aloe* species were not induced diarrhoea and the weight loss impact were minimum compared to the other treatment groups. Therefore, these *Aloe* species were stated as non-toxic in this acute oral toxicity test, which requires further toxicological analysis for safety concern.

The significant difference ($P < .05$) in percentage change in mean absolute body weight and mean final body weight gain of *Aloe harlana*, *A. mcloughlinii*, *A. pirottae* and unidentified/AHU53 showed at both dose levels of 300 mg/kg and 2000 mg/kg body weight could be attributed to potential toxicity coupled with the impact of diarrhoea. Significant level of body weight changes are an indicator of adverse side effects of test substances [45]. In these groups, it was observed that different level of significance different between the doses along the weeks. This could indicate either the dose impact on the body weight loss due to toxic effect or might be the degree of induced diarrhoea resulted in significance difference in body weight loss. In addition, the final body weights of mice administered with exudates of *Aloe pirottae* and *A. harlana* at 2000 mg/kg body weight dose levels were showed less significant difference from the control ($P < .05$) while *Aloe mcloughlinii* and unidentified/AHU53 showed more significant difference ($P < .005$) at same dose level of 2000 mg/kg. This could be attributed to the potential physiological impact of the species rather than dose impact. In this respect, *Aloe mcloughlinii* and unidentified/AHU53 need to be given more safety attention in their oral uses of exudate for treatment of human and livestock ailments. In addition, exudate doses should be an important concern in the wider uses of *Aloe pirottae* and *A. harlana*. These two endemic *Aloe* species were known for diverse uses in

the traditional medicinal uses [32, 33]. In general, the significant difference (* $p < .05$; ** $p < .005$; *** $p < .0005$) in body weight gain in both dose levels at the end of the experimental period compared to the control group signalled safely concern on these four endemic *Aloe* species, which attract further detail analysis.

4.2.2. Relative Organs Weight Changes

It could be postulated that exudates of most *Aloe* species did not induced any toxicity signs on major organs during the test period up to 2000 mg/kg body weight. However, this result is not sufficient to attest to the entire safety of exudates of these *Aloe* species. The percentage of mean relative liver weight in relation to final body weight for *A. harlana* and *A. pirottae* showed no significant difference ($P > .05$) at 300 mg/kg body weight however, significantly different ($P < .05$) at dose of 2000 mg/Kg body weight suggested potential toxic effect of these exudates at higher concentration, which might be fairly inflated the liver. The percentage of mean liver weight in relation to final body weight for *Aloe mcloughlinii* and unidentified/AHU53 found significantly different at both concentrations, 300 ($P = 0.05^*$) and 2000 mg/Kg ($P = 0.005^{**}$) body weight, may suggest potential toxicity of these exudates at both concentrations but more at higher concentration. In reference to the control group, it has been assumed that the liver of treatment groups with *Aloe mcloughlinii* and unidentified/AHU53 might be inflated, which could be a sign of toxicity. The toxic signs observed on liver of treated groups with exudates of *A. harlana*, *A. pirottae*, *A. mcloughlinii* and unidentified/AHU53 were strengthen the need for more attention and safety concern in their therapeutic oral application.

4.3. Food and Water Consumption

The food consumption analysis is important parameter to study the safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and give a good impression of the appropriate response to the treatment [41]. Groups treated with exudates of *Aloe harlana* and *A. pirottae* at 2000 mg/kg body weight and exudates of *Aloe mcloughlinii* and Unidentified/AHU53) at both dose levels of 300 and 2000 mg/kg body weight were found significantly different ($P < .05$). This might be affected the body weight indicating the potential physiological impacts of the test substance from these four *Aloe* species. The food consumption of the rest of the treatment groups were not significantly different, which

has been revealed that it did not adversely affected the basic metabolic processes of mice in these treatment groups so that their final body weight was not much affected compared to the control group. This could indicate the non-toxic effect the test substances in these groups at the dose levels tested. It has been recorded that exudates of all treatment groups did not have physiological impact related to water intake compared to the control group at both dose levels.

5. CONCLUSION

The results indicated that, among the 17 *Aloe* species targeted in this study, 11 species (*A. calidophila*, *A. megalacantha* subsp. *alticola*, *A. megalacantha* subsp. *megalacantha*, *A. otallensis*, *A. pubescens*, *A. rivaie*, *A. rugosifolia*, *A. secundiflora*, *A. tewoldei*, *A. trichosantha* subsp. *longiflora* and *A. yavellana*) could be potentially non-toxic up to 2000 mg/kg body weight of single dose oral applications. Therefore, these 11 Ethiopian *Aloe* species are relatively safe and not harmful if used cautiously in the existing traditional practices at the level tested. In addition, the results demonstrated that most *Aloe* species (except *A. otallensis*, *A. rugosifolia* and *A. trichosantha* subsp. *longiflora*) have been found to be effective as laxative activity in which with lower concentration of 300 mg/kg body weight has been resulted in diarrhoea. This could be due to the presence of aloin A and aloin B in their leaf exudate, which can initiate to conduct phytochemical analysis. The leaf exudate of *Aloe harlana*, *A. pirottae*, *A. mcloughlinii* and unidentified/AHU53 were exhibited low level of toxicity in mice. It might be a liver was fairly changed for mice induced exudates from two potentially toxic aloe species, *Aloe mcloughlinii* and unidentified/AHU53, under average dose of 2000 mg/kg and more significantly different from the control group ($P=.016$ to $.001$). Additional studies to evaluate hepatotoxicity and nephrotoxicity should be carried out in order to provide for the use of exudates from these *Aloe* species as safe medicinal and nutraceutical purposes. More importantly, the two species, *Aloe retrospiciens* and *A. ruspoliana*, were found toxic at lower dose of 300 mg/kg body weight in this study and need to be careful for the identity of wild harvested aloe products while using aloe exudate for any medicinal and cosmetic purposes. In addition, further toxicological studies involving different mechanisms would be helpful to confirm the safety of these *Aloe* species for traditional therapeutic uses, due to an increasing public interest on *Aloe* products in Ethiopia. Moreover, *A. retrospiciens* and *A. ruspoliana* could initiate detail phytochemical and toxicity studies, which has not been done so far, a particular importance of this study.

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APPENDIX

Appendix table 1. Morphological variation among the 23 *Aloe* species covered in this study.

Table 1. Morphological variations among the 23 *Aloe* species



Aloe calidophila



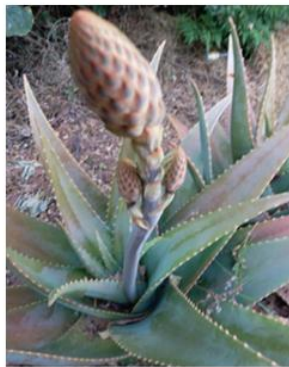
A. megalacantha subs. *alticola*



A. pirottae



A. gilbertii subsp. *gilbertii*



A. pubescens



A. megalacantha subs. *megalacantha*



A. yavellana



A. harlana



A. secundiflora



A. trichosantha subsp. *longiflora*



A. otallensis



A. macrocarpa



A. rivaе



A. citrina



A. rugosifolia



Unidentified-AHU51



Unidentified-AHU53



A. tewoldei



A. mcloughlinii



A. lateritia



A. welmelensis



A. retrospiciens



A. ruspoliana

Appendix table 2. Ethno botany of *Aloe* species by four ethnic community in the study areas (AO=Afan oromo, S=Somali, A=Afar, and H=Harari).

Scientific name	Vernacular name/ Language	Meaning
<i>Aloe calidophila</i>	Hargeessa kormaa/ AO	Male Aloe
	Hargeessa qayaa/ AO	Body fumigation aloe
<i>Aloe citrina</i>	Hargeessa/AO	Aloe
<i>Aloe gilbertii</i> subsp. <i>gilbertii</i>	Hargeessa/AO	Aloe
<i>Aloe harlana</i>	Hargeessa gurachaa/AO	Non-spotted deep green aloe
	Hargeessa buurre gurgudaa/AO	Spotted aloe with large leaf
	Hargeessa buurre xiqaa/AO	Heavily spotted aloe with small leaf
<i>Aloe lateritia</i>	Hargeessa/AO	Aloe
<i>Aloe macrocarpa</i>	Hargeessa hin'adhofnee/AO	Non-bitter aloe
	Set ret/H	Female aloe
<i>Aloe mcloughlinii</i>	Hargeessa/AO	Aloe
	Da'aar Daanyeer/S	Aloe of monkey
<i>Aloe megalacantha</i> subs. <i>alticola</i>	Hargeessa/AO	Aloe
<i>Aloe megalacantha</i> subs. <i>megalacantha</i>	Hargeessa adii/AO	Whitish aloe
	Hargeessa mukaa dheraa/AO	Long stemmed aloe
	Da'aar Dheer/S	Long aloe
<i>Aloe otallensis</i>	Hargeessa/AO	Aloe
<i>Aloe pirottae</i>	Hargeessa buree/AO	Spotted aloe
	Hargeessa baru/AO	Spotted aloe
	Da'aar/S	Aloe
<i>Aloe pubescens</i>	Hargeessa/AO	Aloe
<i>Aloe retrospiciens</i>	Da'aar Maroodi/S	Elephant aloe
	Da'aar dheer/S	Long stem aloe
	Da'aar urso xun/S	Aloe of bad smell
<i>Aloe rivaie</i> Baker	Hargessaa/AO	Aloe
<i>Aloe rugosifolia</i>	Hargeessa Dhalaa/AO	Female Aloe
<i>Aloe ruspoliana</i>	Hargeessa Aja'aa/AO	Aloe of bad smell
<i>Aloe secundiflora</i>	Hargeessa kormaa/AO	Male Aloe
<i>Aloe tewoldei</i>	Hargeessa/AO	Aloe
<i>Aloe trichosantha</i> subsp. <i>longiflora</i>	Hargeessa/AO	Aloe
	Uure/A	Bile or bitter
	Hargessa ilili dheraa/AO	Aloe with long flower stalk
	Ret/H	Aloe
<i>Aloe welmelensis</i>	Hargeessa/AO	Aloe
<i>Aloe yavellana</i>	Hargeessa/AO	Aloe
AHU53- unknown	Hargeessa Jawwe/AO	Aloe of snake
	Hargeessa buburre xiqqa/AO	Spotted small aloe
AHU51- unknown	Hargeessa laafaa/AO	Soft aloe

Appendix table 3. The population status from emic perspectives, some new records of flowering period, and collection sites of *Aloe* species.

Species	IUCN Category/ Population trend [17-20]	Population status from informants (FC%±SD)	Flowering period [5] with new records	Floristic regions of natural distribution/Collection localities
<i>Aloe calidophila</i>	LC/Decreasing	Decreasing (64.5 ± 4.2)	September to December	SD/Alona kebele on the way to Arero, Dida Chena plain between Yabelo and Mega of Borena
<i>Aloe citrina</i>	LC /Unknown - Population severely fragmented	Decreasing (55 ± 3.1)	September to December & May to June	BA/near Sof-Omor
<i>Aloe gilbertii</i> subsp. <i>gilbertii</i>	LC /Increasing	Stable (52.2 ± 3.7)	October to December	AR/ Wayo Hargessa or Rafu Hargessa kebele in Arsi Negele
<i>Aloe harlana</i>	EN/Decreasing	Highly decreasing (88.4 ± 2.6)	April to May, also from September to October/ November in garden for spotted type	HA/ ^s Dengego mountains and valley complex/Harla village
<i>Aloe lateritia</i>	LC /Unknown	Decreasing (65.2 ± 4.4)	September to November	SD/ Goro Qalqalchaa Kebele of West Guji zone
<i>Aloe macrocarpa</i>	LC /Stable	Stable(56 ± 3.8)	October to April/November in the garden	HA, AF, BA, AR, SD/ ^s Dengego mountain of Harla village and Hakim mountain of Harar
<i>Aloe mcloughlinii</i>	LC /Decreasing	Stable(58 ± 2.2)	April to May/November in wild; December, January, February, July in garden	HA/ Melka Jebdu of Dire Dawa and ^s Dengego mountain of Harla village
<i>Aloe megalacantha</i> subs. <i>alticola</i>	No data/No data	Decreasing(64 ± 2.6)	August to October, sometimes April	HA/Chelenko Hara and Kobo of east Hararge
<i>Aloe megalacantha</i> subs. <i>megalacantha</i>	LC /Decreasing	Stable(52 ± 5.8)	August to October, sometimes April to May/July, November	HA, BA/ Dusmo kebele Yu'ale, ^s Dengego mountain and on the way to Sof Omor
<i>Aloe otallensis</i>	LC /Stable	Stable(54 ± 4.6)	September to December/ Sometimes May	SD/ Goro Qalqalchaa Kebele of West Guji zone
<i>Aloe pirottae</i>	LC /unknown	Decreasing (68.4 ± 5.1)	November to January	HA, BA/ Goro on the way to Sof Omor and Erer valley of Babile
<i>Aloe pubescens</i>	NT/Stable	Decreasing (58.5 ± 2.2)	February to May/ December in garden	HA/Chelenko hara and Karamile mountain, east Hararge

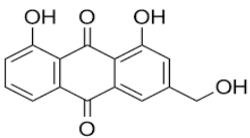
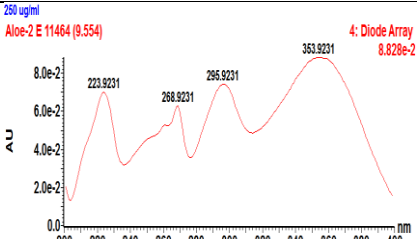
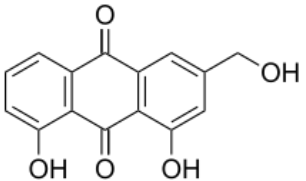
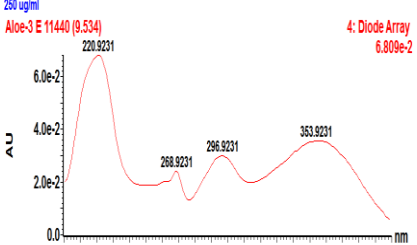
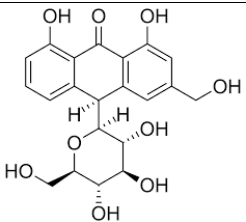
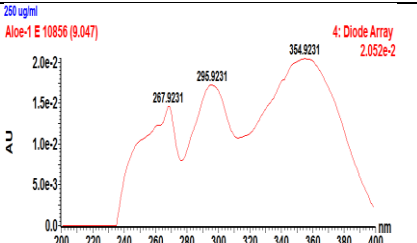
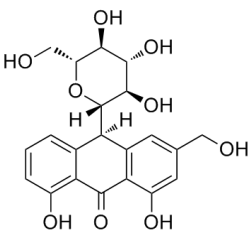
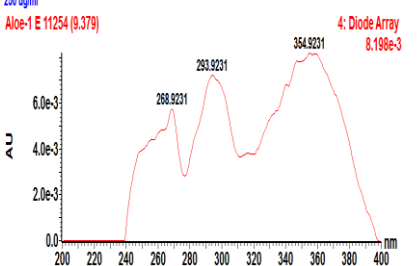
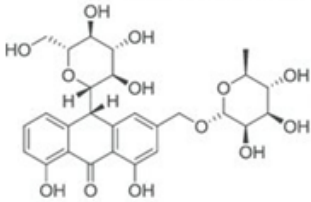
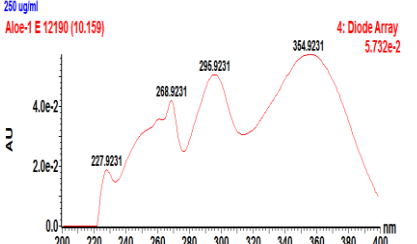
<i>Aloe retrospiciens</i>	VU/unknown	Decreasing (78.2 ± 2.1)	March to April & September to December Φ February in garden	HA, AF/ Geilsa kebele in Amibara and Degha Chabs kebele in Shinile of Somali region
<i>Aloe rivaie</i>	LC /Decreasing	Decreasing (56.5 ± 3.4)	September to December/ Φ May in some population	SD/ 18 kms before Mega while coming from Yabelo
<i>Aloe rugosifolia</i>	VU/Decreasing	Decreasing(62.4 ± 4.6)	July to September	SD/ Dida Chena plain between Yabelo and Mega
<i>Aloe ruspoliana</i> *	LC /Decreasing	Decreasing (64.8 ± 1.8)	September to December	BA/ Kersa kebele near Goro and Cliff of Sof omor Cave
<i>Aloe secundiflora</i>	LC/Stable	Stable(51.6 ± 6.8)	April to May & August to December	SD/ Dida Chena plain between Yabelo and Mega
<i>Aloe tewoldei</i>	NT/Unknown	Decreasing (69.2 ± 6.2)	October in cultivation	BA/ Kersa kebele near Goro
<i>Aloe trichosantha</i> subsp. <i>longiflora</i>	LC/Stable	Increasing (52.6 ± 8.2)	Almost throughout the year	HA, AF, BA/ Uure letti of Afar, Haramaro Deyma kebele of west Hararge, § Dengego mountain top, Lake Beseka costal
<i>Aloe welmelensis</i>	DD/Unknown	Decreasing (51.5 ± 3.1)	December	BA/ along Welmel rivers
<i>Aloe yavellana</i>	EN/Decreasing	Decreasing (64.2 ± 1.4)	September to October	SD/ near Yabelo
Unidentified (AHU51)	Expected to be new species	Highly decreasing (72.2 ± 1.8)	Φ January, February, March, April, May, June, July, August, October, November	HA/ § Dengego mountains & valleys
Unidentified (AHU53)		Highly decreasing (84.8 ± 1.6)	Φ October, March, April, May, July, August, January, in garden	HA/ Gorro Gaadaa kebele in Melka-Jebdu and top of § Dengego mountains

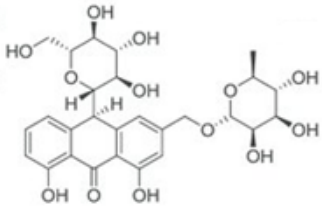
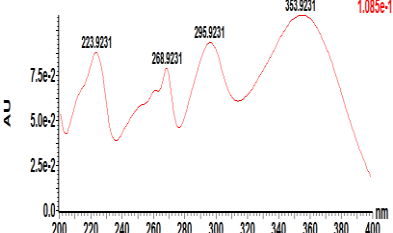
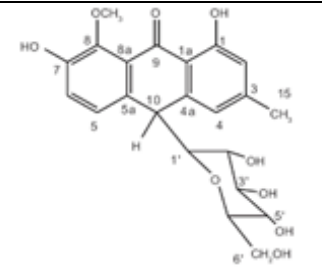
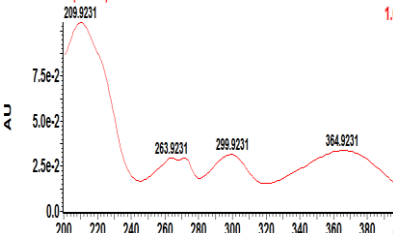
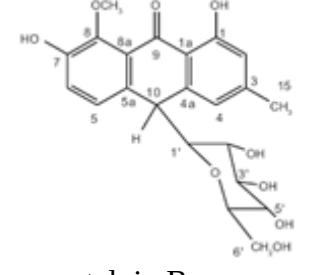
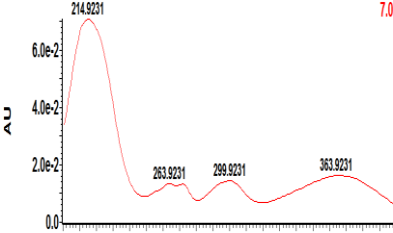
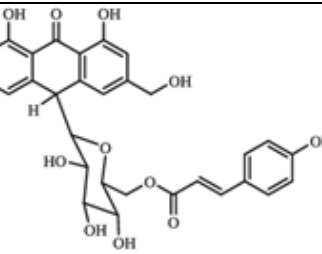
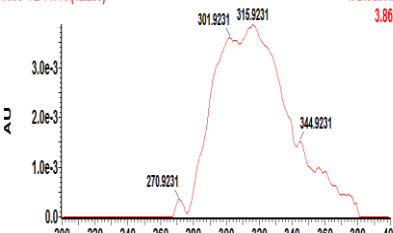
*new record 1777 masl altitude, at the bushland plain field in Kersa kebele (smallest administrative unit) near Goro of Bale Floristic region

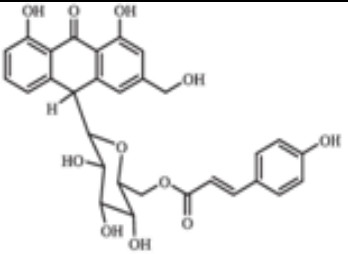
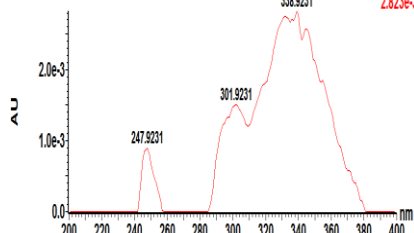
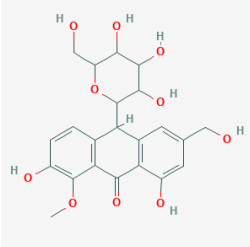
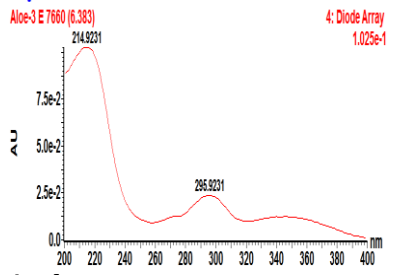
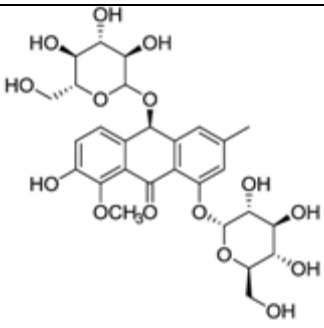
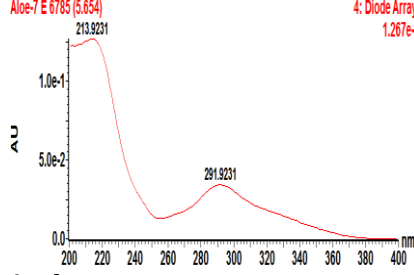
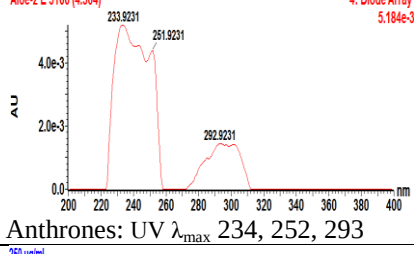
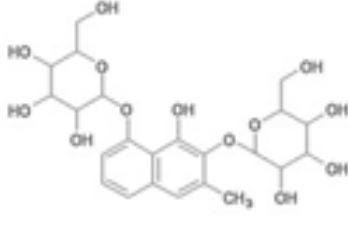
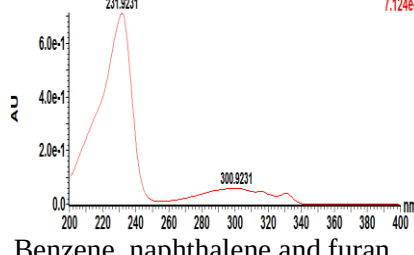
Φ Newly recorded flowering months other than literature reports; FC = frequency of citation by informants in percentage and SD = Standard deviation LC = least concern; EN = endangered; DD = data deficient; NT = near threatened; VU = vulnerable; Hararge (HA), Bale (BA), Sidamo (SD), Arsi (AR), and Afar (AF) Floristic Regions

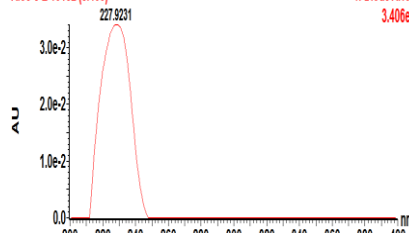
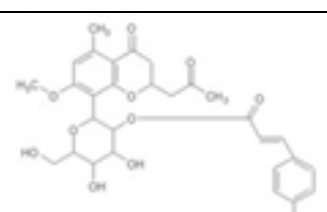
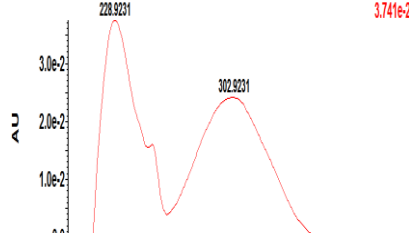
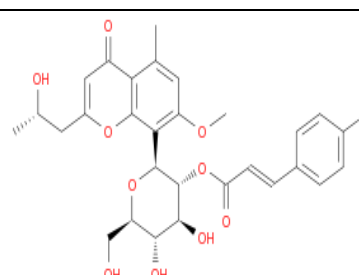
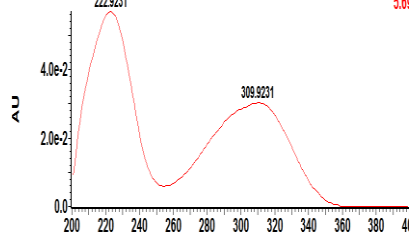
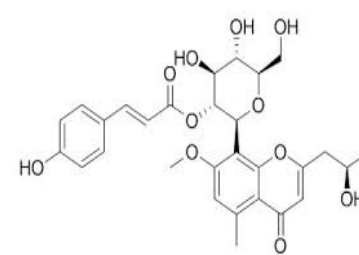
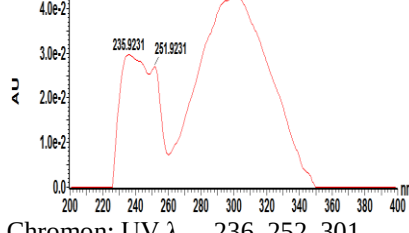
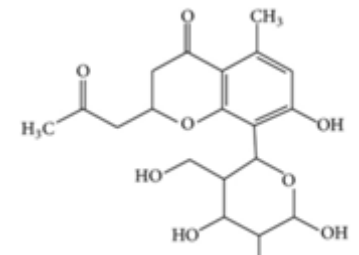
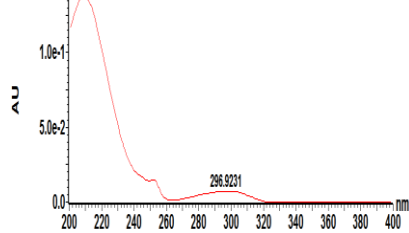
§ Dengego Mountains & Valleys complex is unique ecosystem for *Aloe* complex study, which harbored about seven *Aloe* species and potentially more infraspecific taxa due to the potential natural hybridization of the species in this genus.

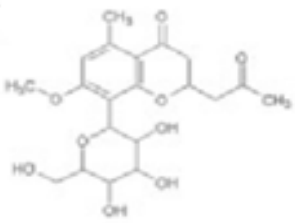
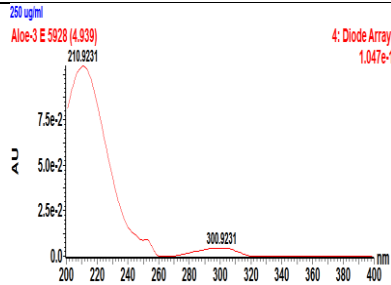
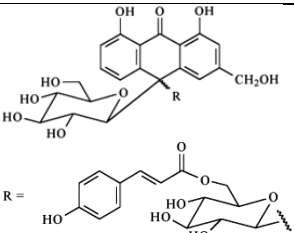
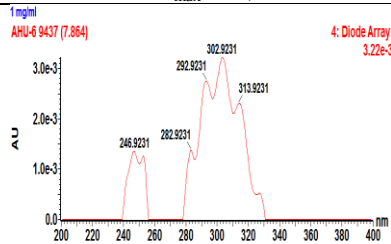
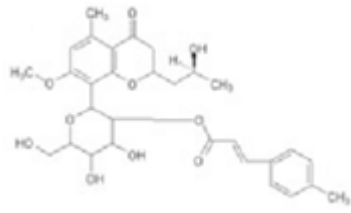
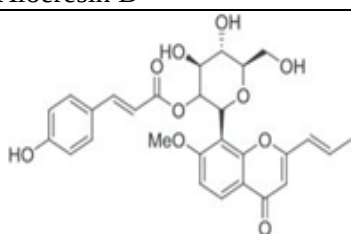
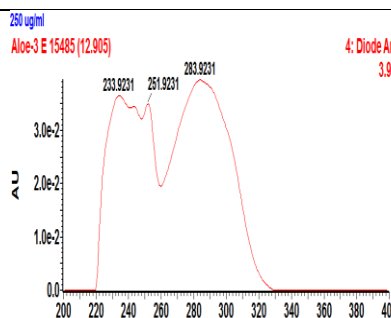
Appendix table 4. List of the major identified compounds with their retention time (Rt) and Pseudomolecular ion in m/z ([M-H]⁻), MS fragmentation (m/z), compound name and chemical structure, DAD UV spectrum and compound class

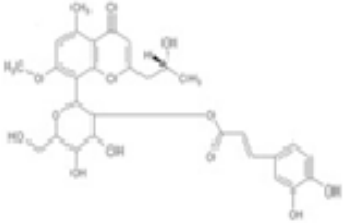
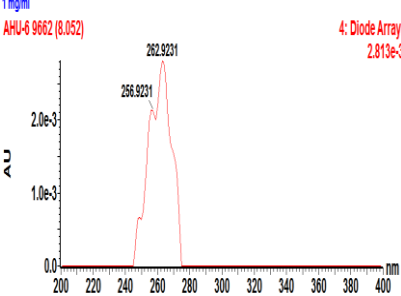
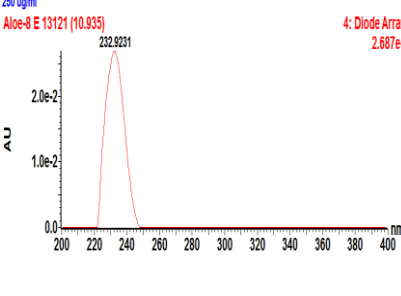
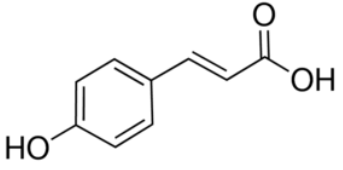
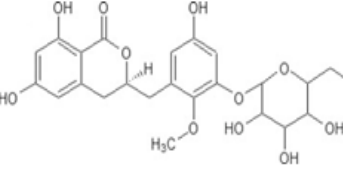
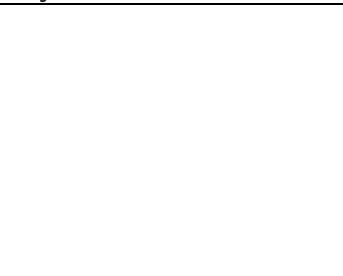
Rt/ [M-H] ⁻	Fragmentation (m/z)	Name and Structure	UV spectrum and compound class
7.114/ 461.1071	407, 339.8, 293, 261, 243.8, 215, 211, 183, 151	 Aloe-emodin	 Aloe-2 E 11464 (9.554) 4: Diode Array 8.828e-2 Anthraquinone
7.149/ 475.0853	431, 407, 293, 243.8, 211, 155, 151	 Aloe-emodin-8-O-glucoside (5-Hydroxyaloin-6'-acetate)	 Aloe-3 E 11440 (9.534) 4: Diode Array 6.809e-2 Anthrone: UV λ _{max} : 221, 269, 296, 358 nm
9.114/ 417.1187	407, 299, 298, 297, 261, 151	 Aloin A	 Aloe-1 E 10856 (9.047) 4: Diode Array 2.052e-2 Anthrone UV λ _{max} 268, 296, 355
9.608/ 417.1190	299, 298, 297, 211	 Aloin B	 Aloe-1 E 11254 (9.379) 4: Diode Array 8.199e-3 Anthrone UV λ _{max} 269, 294, 355
10.227/ 563.1772	445, 444, 443, 406.9, 292.9, 261, 210.9	 Aloinoside A	 Aloe-1 E 12190 (10.159) 4: Diode Array 5.732e-2 Anthrone UV λ _{max} 228, 269, 296, 355

9.466/ 563.1763	445, 444, 443, 407, 293, 261, 215		250 µg/ml Aloe-1 E 11464 (9.554)  4: Diode Array 1.085e-1 Anthrone UV λ_{max} 224, 268, 296, 354
10.506/53 3.1652	445, 444, 443, 341, 340, 293, 243.8, 183, 151		Not detected Anthrone
10.33/ 431.0988	313, 312, 311, 261, 151		250 µg/ml Aloe-9 E 12325 (10.271)  4: Diode Array 1.047e-1 Anthrone UV λ_{max} 264, 300, 365
9.853/ 431.0988	313, 312, 311, 267, 243.8		250 µg/ml Aloe-9 E 11769 (9.808)  4: Diode Array 7.079e-2 Anthrone UV λ_{max} 215, 264, 300, 364
12.488/ 563.1589	443, 399, 297, 261		250 µg/ml Aloe-4 E 14710 (12.259)  4: Diode Array 3.863e-3 Anthrone UV λ_{max} 271, 302, 316, 345

12.398/ 563.1602	444, 443, 399, 261, 151		250 µg/ml Aloe-4 E 13579 (11.316)  4: Diode Array 2.823e-3 Anthrone UV $\lambda_{\max}$ 248, 302, 339
6.444/ 447.1284	406.9, 328, 327, 284, 261		250 µg/ml Aloe-3 E 7660 (6.383)  4: Diode Array 1.025e-1 Anthrone UV $\lambda_{\max}$ 215, 296
5.70/ 593.1878	531, 475, 474, 473, 431, 407, 311, 261, 151		250 µg/ml Aloe-7 E 6785 (5.654)  4: Diode Array 1.267e-1 Anthrone UV $\lambda_{\max}$ 214, 292
4.342/ 395.1328	351, 339.8, 275, 231, 183, 151		250 µg/ml Aloe-2 E 5166 (4.304)  4: Diode Array 5.184e-3 Anthrones: UV $\lambda_{\max}$ 234, 252, 293
7.205/ 513.1625	352, 351, 211, 189, 167		250 µg/ml Aloe-8 E 8559 (7.132)  4: Diode Array 7.124e-1 Benzene, naphthalene and furan derivatives: UV $\lambda_{\max}$ 232, 301

8.512/ 527.1767	459, 408, 407, 365, 293, 261, 183, 151	1- <i>O</i> -methylpicaloside	250 µg/ml Aloe-8 E 10152 (8.460) 4: Diode Array 3.406e-2  UV spectrum showing a single peak at 227.9231 nm. The y-axis is AU (0.0 to 3.0e-2) and the x-axis is nm (200 to 400). Benzene, naphthalene and furan derivatives: UV λ_{max} 228
9.552/ 553.1724	511, 408, 407, 293, 261	 7-<i>O</i>-Methylaloesin A	250 µg/ml Aloe-7 E 11375 (9.479) 4: Diode Array 3.741e-2  UV spectrum showing two peaks at 228.9231 nm and 302.9231 nm. The y-axis is AU (0.0 to 3.0e-2) and the x-axis is nm (200 to 400). Chromon: UV λ_{max} 229, 303
10.506/ 555.1509	541, 431, 391, 308, 307, 247, 243.8	 Isoaloesin D	250 µg/ml Aloe-9 E 12566 (10.472) 4: Diode Array 5.696e-2  UV spectrum showing two peaks at 222.9231 nm and 309.9231 nm. The y-axis is AU (0.0 to 4.0e-2) and the x-axis is nm (200 to 400). Chromon: UV λ_{max} 223, 310
9.131/ 555.1874	511, 419, 418, 417, 299, 298, 297, 261, 145	 Aloeresin D	1 mg/ml AHU-6 11102 (9.252) 4: Diode Array 4.261e-2  UV spectrum showing three peaks at 235.9231 nm, 251.9231 nm, and 300.9231 nm. The y-axis is AU (0.0 to 4.0e-2) and the x-axis is nm (200 to 400). Chromon: UV λ_{max} 236, 252, 301
4.11/ 393.1183	339.8, 273, 243, 151	 Aloesin	250 µg/ml Aloe-3 E 4870 (4.057) 4: Diode Array 1.368e-1  UV spectrum showing two peaks at 208.9231 nm and 296.9231 nm. The y-axis is AU (0.0 to 1.0e-1) and the x-axis is nm (200 to 400). Chromon: UV λ_{max} 209, 297

4.961/ 407.1358	406.9, 347, 261, 215, 183, 151		 250 ug/ml Aloe-3 E 5928 (4.939) 4: Diode Array 1.047e-1 Chromon: UV $\lambda_{\max}$ 211, 301
7.91/ 539.1559	433, 407, 376, 375, 293, 261, 163	 6'-O-Coumaroyl aloesin	 1 mg/ml AHU-6 9437 (7.864) 4: Diode Array 3.22e-3 Chromon: 247, 283, 293, 303, 314
8.404/ 505.1332	408, 407, 344, 343, 293, 261, 215, 183, 151		Not detected Chromon
12.95/ 537.1757	390, 389, 311, 261, 215, 147		 250 ug/ml Aloe-3 E 15485 (12.905) 4: Diode Array 3.95e-2 Chromone: UV $\lambda_{\max}$ 234, 252, 284
8.546/ 569.1648	459.8, 407, 341, 311, 293, 243.8, 215, 183		Not detected Chromone:

8.142/ 571.1840	505, 407, 343, 293, 261, 215,183	 Isorabaichromone	 Chromon: UV $\lambda_{\max}$ 257, 263
11.005/ 583.2023	495, 423, 422, 421, 407, 333, 293, 261, 151	 7-O-Methyl-2'-feruloylaloetin	 Chromon: UV $\lambda_{\max}$ 233
11.568/ 651.1706		 p-Coumaric acid derivatives	Not detected Coumarin
5.734/ 525.1601	459.8, 408.8, 407, 293, 261, 215, 183, 151	 Dihydroisocoumarin	Not detected Coumarin
11.233/ 343.0811	299, 24308, 183, 151	 5,3'-Dihydroxy-6,7,4'- trimethoxyflavone(eupatorin)	Not detected Flavonoid

11.357/ 579.1499	577, 460, 459, 407, 311, 293, 261, 243.8, 215, 183, 151	Kaempferol-3-O-hexosyl-O- pentoside	Not detected Flavonoid
4.604/ 329.0333	329, 293, 261, 243.8, 183, 151	Trihydroxy octadecenoic acid	Oxilipin: UV $\lambda_{\max}$ 235
7.325/ 409.1138	293, 248, 247, 243.8, 243.8, 183, 151	Aloenin	Pyrans and pyron: 205, 296
8.12/ 505.1354	408, 407, 344, 343, 293, 261, 183, 211, 183, 151	3,4-Dihydro-6,8-dihydroxy- 3-(2'-acetyl-3'-O- <i>b</i> -D- glucopyranosyl-5'- hydroxyphenyl) methyl- 2(1H)-benzopyran-1-one	Not detected Pyrans and pyrones
8.103/ 717.2029	555, 436, 409, 307, 211	Aloenin B	Pyrans and pyrones: 201, 309

Appendix table 5. List of chemicals and standards used in the HPTLC and UHPLC-MS-DAD techniques.

Chemicals	Uses	Amount used		Description
		In HPTLC	In UHPLC	
ethyl acetate/EtOAc (C ₄ H ₈ O ₂)	HPTLC Mobile phase	90 ml	-	Mixed together in a given volume
acetonitrile (CH ₃ CN)		20 ml	100%	
formic acid (CH ₂ O ₂)		10 ml	0.1%	
methanol/MeOH (CH ₃ OH)	Dilution and rinsing	30 mL	-	Dilution of exudate and rinsing the syringe
chloroform/trichloromethane (CHCl ₃)	Cleaner for derivatizer	-	250 µL for UHPLC	Used after derivatization
potassium hydroxide (4% KOH)	Component of derivatized	-	-	derivatizer
2-aminoethyl diphenyl borinate (1.5gm)	Component of derivatized	1.5gm in 150 ml MeOH	-	natural product derivatizer preparation
polyethylene glycol 6000 (7.5 gm)	Component of derivatized	7.5 gm in 150 ml ethanol	-	natural product derivatizer preparation
Aloin A	Standard	2 µL	-	Pure standards from suppliers
Aloin B	Standard	2 µL	-	
Aloinoside A	Standard	2 µL	-	
Aloinoside B	Standard	2 µL	-	
Microdentin A	Standard	2 µL	-	
Microdentin B	Standard	2 µL	-	
Homonataloin A	Standard	2 µL	-	
Homonataloin B	Standard	2 µL	-	
Dihydroisocoumarin	Standard	2 µL	-	Isolated from south African Aloes
Aloeresin A	Standard	2 µL	-	
Aloeresin D	Standard	2 µL	-	
Aloeresin G	Standard	2 µL	-	

