POLYPHENOLIC ANALYSIS AND ANTIOXIDANT PROPERTIES OF THE ETHIOPIAN SPICES, *Foeniculum vulgare* Mill. And *Syzygium aromaticum* (L.) Merr. & Perry

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Polyphenolic Analysis and Antioxidant Properties of the Ethiopian Spices, *Foeniculum vulgare* Mill. and *Syzgium aromaticum* (L.) Merr. & Perry

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POSTGRADUATE PROGRAM DIRECTORATE

We hereby certify that we have read and evaluated this thesis entitled "**Polyphenolic Analysis and Antioxidant Properties of the Ethiopian Spices, *Foeniculum vulgare* Mill. and *Syzgium aromaticum* (L.) Merr. & Perry"** prepared, under our guidance, by **Shibiru Temesgen**. We recommended that it is submitted as fulfilling the thesis requirement.

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DEDICATION

I dedicate this thesis manuscript to my father, Temesgen Buli, my mother, Agitu Nemera and my brother Ifa Temesgen for their encouragement.

STATEMENT OF AUTHOR

First, I declare that this thesis is my own work and that all sources of materials used for the thesis have been accordingly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for M.Sc. Degree in Biotechnology at the Haramaya University and is deposited at the University Library to be made available to borrowers under rules of the Library. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author Shibiru Temesgen Buli was born in Wenjo town, West Wollega, Oromia Regional State on January 01, 1993. He attended his elementary education in Wenjo Elementary School and went to Enango High School to attend his secondary school. He completed Secondary education in Enango and went to Wollega Adventist Academy (WAA) to follow his preparatory school. Then, he joined Dire Dawa University in 2009 and graduated with B.Sc. in the field of Applied Biology in July 2011 G.C. After that, he was employed by Haramaya University Chiro Campus as Graduate Assistant I and II and assistant lecturer, where he worked for four years and still serving until, he joined the Post Graduate Program at Haramaya University to pursue his MSc degree in Biotechnology.

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ACRONYM AND ABBREVIATION

AA	Ascorbic Acid
BHA	Butyl Hydroxy Anisole
BHT	Butyl Hydroxy Toluene
CE	Catchetin Equivalent
DPPH	Diphenyl Picryl Hydrazyl
DW	Dry Weight
EBTS	EthylBenzoThiazoline-6-Sulphonic acid
ROS	Reactive Oxygen Species
RP-HPLC	Reversed Phase High Performance Liquid Chromatography
TBARS	Thiobarbutric Acid Reactive Substance
TEAC	Trolox Equivalent Antioxidant Capacity
TFC	Total Flavonoid Compound
TPC	Total Phenolic Compound

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ABSTRACT

Natural products, especially those of spices origin, have always been an important source of therapeutic agents against chronic oxidative stress-related diseases. This study was aimed to analyse polyphenolic metabolites and antioxidant activity of Ethiopian Clove or kirunfud (Syzygium aromaticum (L.) Merr.) and *Fennel or ensilal (Foeniculum vulgare Mill.) extracts. Seeds of fennel and flower of clove were purchased and extraction was done using chloroform, ethyl acetate, methanol and water solvents in triplicate. Qualitative identification analysis of the obtained extracts was performed using standard procedure, and quantitative estimation of representative polyphonic content were determined using spectrophotometer technique at different UV light. Antioxidant capacity of extracts of the spices was determined using Dipenyl Picryl Hydrazyl (DPPH), nitric oxide, hydrogen peroxide scavenging and reducing power assays. The result showed that, the phenols and flavonoids were predominantly presented in more polar solvent extracts of fennel and clove. The aqueous extracts of both spices showed significantly the highest total phenolic compound. Methanolic fennel and aqueous clove extracts showed significantly higher total flavonoid content. Antioxidant activity tests showed that ethyl acetate fennel extracts showed the highest DPPH scavenging at all concentrations compared to the standard Butyl Hydroxy Toluene (BHT). Chloroform fennel extracts showed the highest NO^- radical scavenging activity at all concentration compared to the BHT and other extracts, except at 400 µg/mL, and chloroform clove extracts showed the highest NO^- radical scavenging activity at all concentration compared to the standard (BHT) and other extracts, except at 25 µg/mL. Among the fennel and clove spice solvent extracts, the potent H_2O_2 free radical scavenger was methanolic extract at all concentrations, except at 400 µg/mL for fennel and 25 µg/mL for clove. For fennel, the best reducing power was showed by ethyl acetate extract for clove, the best reducing power was showed by methanolic extract. The study showed that the extracts have the proton – donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. In vivo antioxidant activity and isolation of antioxidant components of the tested spices are recommended to be carried out.*

Keywords: Spices; antioxidant activity; Qualitative; Total flavonoid

1. INTRODUCTION

Plant polyphenols are secondary metabolites that are not required for normal growth and development of plants. In plant kingdom they are limited in occurrence and may be restricted to a particular taxonomic group: genus, species or family (Irchhaiya *et al*., 2015). Most of polyphenolic metabolites have health benefits through their reactive oxygen species (ROS) scavenging properties. Free radical and related species are generated in the body as a result of metabolic reactions. Accumulation of free radicals causes damages in living systems resulting in oxidative stress (Khanta and Mohammad, 2011). The free-radical scavengers (antioxidants) have potential to prevent, delay many of human chronic and ageing diseases such as cancer, diabetes, heart disease, stroke, malaria and rheumatoid arthritis. Free radical scavenging is an important mechanism for the inhibition of lipid peroxidation, and can be a good marker for antioxidant activity (Mann, 2011).

Many scientific sources show that most plants like spices and herbs are rich in secondary metabolites including polyphenolic metabolites, terpenes, phenolics and Nitrogen and Sulfur containing compound (Khanta and Mohammad, 2011). For example, high level of phenolic metabolites in methanol extract of leaves, rhizomes and stems in different varieties of *Syzygium aromaticum* was reported by Ali *et al.* (2010). In addition to health benefits, spices are common food adjuncts, which have been used as flavoring, seasoning, and coloring agents and sometimes as preservatives throughout the world for thousands of years which make it favorable for consumption **(**Yizhong *et al.,* 2005). Therefore, spices are part of the daily food in several parts of the world. It is being used as food additives mostly for their organoleptic attributes. It is now understood that spices also exhibit several beneficial physiological effects in addition to enhancing taste and flavor of food (Ali *et al.,* 2015). The digestive stimulant action of spices has been well recognized for a long time, and a few of them find pharmacological application against digestive disorders. Hence, Consumption of spices has been implicated in the prevention of cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis (Srinivasan, 2005).

Clove (*Syzgium aromaticum* is a branched rhizome that has a characteristic aromatic and pungent flavor belonging to the Zingiberaceae family. It has been cultivated since ancient times for cooking and medicinal preparations (Leal *et al.,* 2003). *Syzygium aromaticum* is known to be a traditional medicinal plant used as an expectorant, anti-emetic, stimulant, anti-flatulent and for treatment of

dyspepsia. It is also used as an anodyne and antiseptic in dentistry (Raina *et al*., 2001). Clove extracts are rich in Polyphenolic that exhibit antioxidant activity; researchers have demonstrated that other substances present in clove extracts inhibit the growth of Mycobacterium and *Mycobacterium tuberculosis* (Leal *et al*., 2003).

The *Foeniculum vulgare*, known as fennel, has a long history of herbal uses as both food and medicine (Abebie *et al*., 2017 A and B; Yaldiz and Camlica, 2019). A compiled review article indicates fennel efficacy in several *in vitro* and *in vivo* pharmacological properties such as anti-microbial, anti-viral, anti-inflammatory, anti-mutagenic, anti-pyretic, anti-spasmodic, anti-thrombotic, apoptotic, cardiovascular, chemo modulatory, anti-tumor, hepatoprotective, hypoglycemic, hypolipidemic, and memory enhancing property related to digestive, endocrine, reproductive, and respiratory systems. In general, it has been used for more than forty types of disorders (Shamkant *et al*., 2014).

Previous study revealed that anti-oxidant and anti-microbial activities of the crude extract of *Foeniculum vulgare* extracted by different solvents indicating a high potential to be used as natural antioxidants in food preservation as well as for preventing oxidative stress mediated human disorders (Abebie *et al*., 2017 A and B; Yaldiz and Camlica, 2019). Aqueous: methanol (20:80, v/v) extract of leaf contained the highest TPC [(24.3 ± 0.99) mg GAE/g], and TFC [(18.92 ± 0.34) µg CE/mg] and showed the highest DPPH radical scavenging activity (IC50 = 69.68 ± 2.28 µg/mL). The aqueous: methanol (20:80, v/v) extract had strongest reducing ability (0.82 ± 0.06 nm). Chloroform extract exhibited the highest total antioxidant capacity (1.94 ± 0.76 mg AAE/g) as determined by the phosphomolybdenum method. Except for total antioxidant activity, all antioxidant activities were positively correlated TPC and TFC (Abebie *et al*., 2017 A). Methanol extracts of seed contained the highest TPC [(22.93 ± 7.17) mg GAE/g], and TFC [(8.581 ± 1.22) µg CE/mg] and water extract showed the highest DPPH radical scavenging activity (IC50 = 207.94 ± 83.38 µg/mL) (Abebie *et al.,* 2017 B).

Ethiopia is endowed with enormous biodiversity resources. The floral wealth is diverse and many of them could be food or remedy for the native people. Many of the native plants are used in Ethiopia for daily consumption in the form of spices. They are either cultivated or found in the wild. Ethiopia is characterized by diverse climatic conditions, conducive for the growth of different vegetables known for their nutritional values. Nevertheless, studies on scientific validation of the vegetables, spices and fruits in Ethiopia are scanty. Previously, three Ethiopian *Rubus* species were evaluated for their antioxidant activities (Tadesse Tesfaye *et al*., 2007). The metal chelating activity, antioxidant properties, and the effect on carbohydrate-hydrolyzing enzymes of Ethiopian spice blend Berbere have also been investigated. Berbere contains a total amount of phenols corresponding to 71.3 mg chlorogenic acid equivalent per gram of extract and a total flavonoid content of 32.5 mg quercetin equivalent per gram of extract **(**Loizzo *et al*., 2011**)**.

However, studies on polyphenolic metabolites' profile/content and their anti-oxidant activities of spices grown and consumed in Ethiopia are scanty. Moreover, a food-based research would help us to elucidate more health effects of various plant components. Therefore, it is of great interest in research to elucidate the Polyphenolic metabolites like Polyphenols content and their antioxidant properties in spices consumed in Ethiopia. This study was, therefore, designed to investigate the polyphenolic content of selected spices extracts and evaluate their antioxidant properties *in vitro* with the following objectives.

General objective

To analyse polyphenols contents and their antioxidant capacity of Clove (*Syzygium aromaticum* and Fennel (*Foeniculum vulgare*).

Specific objectives

- To identify and analyse the polyphenolic quality of the extracts of the selected spices
- To estimate total representative polyphenolic content of the extracts of the selected spices
- To evaluate antioxidant properties of the selected spices extracts

2. LITERATURE REVIEW

2.1. Plant Secondary Metabolites

A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins and tannins etc). They act as defense chemicals. Their absence does not cause bad effects in the plants (Leonie and Klaas, 2011). The secondary metabolites are metabolites that are not required for normal growth and development, and are not made through metabolic pathways common to all plants. In plant kingdom they are limited in occurrence to a particular taxonomic group: genus, species or family (Irchhaiya *et al*., 2015).

Most of secondary metabolites have health benefits through their reactive oxygen species (ROS) scavenging properties. Free radical and related species are generated in the body as a result of metabolic reactions. As Cao *et al.,* (1997), Accumulation of free radicals causes damages in living systems resulting in oxidative stress. The free-radical scavengers (antioxidants) have potential to prevent, delay many of human chronic and ageing diseases such as cancer, diabetes, heart disease, stroke, malaria and rheumatoid arthritis (Mann, 2011). Free radical scavenging is an important mechanism for the inhibition of lipid peroxidation, and can be a good marker for antioxidant activity.

Many scientific sources state that their role is not crucial for living cells in normal growth, development, and reproduction but they act in defense purposes to protect a plant from any possible harm in the ecological environment and other interspecies protection (Cao *et al.,* 1997). Therefore, they are usually synthesized in plants for particular needs, while the primary metabolites have generally the shared biological purposes across all species (Khanta and Mohammad, 2011). Secondary metabolites may often be created by modified synthetic pathways from primary metabolite. Plants have been evolving to adapt to the environment with genetic encoding of useful and diverse synthesis for secondary metabolites. In human life, these metabolites are used as medicines, flavorings, or relaxing drugs, especially essential oils (Edmond, 2014).

There are three major groups of secondary metabolites (terpenes, phenolic and Nitrogen and Sulfur containing compound). Terpenes are composed of 5 carbon isopentanoid units, are toxins and feeding deterrents to many herbivores (Wettasinghe and Shahidi, 1999). Phenolics synthesized primarily from products of the shikimic acid pathway, have several important defensive roles in the plants. Members of the third major group i.e. N and S containing metabolites are synthesized principally from common amino acids (Khanta and Mohammad, 2011).

Researches show that most plants used as spices are rich in secondary metabolites, though the type and amount vary with plant species. One of the benefits of culinary herbs and spices are primarily due to this antioxidant property. Free radical and related species are generated in the body as a result of metabolic reactions. Accumulation of free radicals causes damages in living systems resulting in oxidative stress (Wettasinghe and Shahidi, 1999). The free-radical scavengers (antioxidants) have potential to prevent, delay or improve many of human chronic and ageing diseases such as cancer, diabetes, heart disease, stroke, malaria and rheumatoid arthritis. Free radical scavenging is an important mechanism for the inhibition of lipid peroxidation, and can be a good marker for antioxidant activity; results indicate that the addition of some spices and herbs to food products can prevent their oxidative deterioration (Mann, 2011).

2.2. Polyphenolic Antioxidants

Phenolic metabolites can retard lipid oxidation by donating a hydrogen atom or an electron to chain-initiating free radicals such as the hydroxyl and superoxide radicals (Cao *et al.,* 1997; Silva *et al*., 1991 as cited in Wettasinghe and Shahidi, 1999). They can also neutralize the substrate-derived free radicals such as the fatty acid free radicals and alkoxy radicals. This property of plant extracts has an important role in retarding lipid oxidation in food products and living tissues. Incorporation of such extracts in human foods not only preserves the wholesomeness of the food but also reduces the risk to humans of developing atherosclerosis and cancer. Some phenolic metabolites present in plant extracts are reported to retard lipid oxidation through chelating transition metal ions such as those of iron, copper, and manganese (Wettasinghe and Shahidi, 1999).

Phenolics are able to terminate free radicals and chelate metal ions that are capable of catalyzing formation of oxygen reactive species that promote lipid peroxidation (Wettasinghe and Shahidi, 1999). Phenolic compound interferes with oxidation of lipids and other free radicals by rapid donation of hydrogen atom or electrons to the oxidized molecules or radicals. The resultant radicals from the reaction of phenols with lipid radical are stabilized by the decolorization of unpaired electrons around the aromatic ring (Ingold, 1960). Stability of the phenoxy radical reduces the rate of propagation of auto-oxidation chain reactions because the propagation reaction is slow and the bulky groups of 2 and 6 positions offers steric hindrances in the region of the radical and reduces the rate of propagation (Hudson, 1990). Phenolic metabolites are understood to induce the cellular antioxidants system; quercetin and flavonoid were found to increase the intra cellular concentration of the glutathione by approximately 50% (Muchuweti *et al*., 2007).

The latest investigations on Phenolic metabolites suggested that cellular effects of flavonoid may be mediated by their interactions with specific proteins central to intracellular signaling cascades. Thus, flavonoid may act as signaling molecules (Wettasinghe and Shahidi, 1999). Phenolic metabolites cannot be produced by the human body and thus must be taken in mainly through the daily diet. Knowledge about the nutritional and therapeutic role of dietary phenolic antioxidants is essential for the development of functional foods, which refers to the improvement of conventional foods with added health benefits. On the other hand, detailed chemical composition of foods considered to be functional is needed, and the main goal of the chemistry of natural metabolites is screening for promising biologically active substances of plant origin. Pepper fruits (*Capsicum annuum* L.) are important vegetables used as vegetable foods and as the spice. Peppers are a good source of vitamins C and E as well as provitamin A **(**Ałgorzata *et al*., 2005**)**.

2.3. Antioxidant Properties of Spices

Spices have been investigated for their antioxidant properties for many years back. As early as 1952, many spices were examined and 32 spices were found to retard the oxidation of lard (Yizhong *et al.,* 2005). Many studies indicated that rosemary, sage, oregano, and thyme, leafy spices in the family labiatae, demonstrated high antioxidant activity. Several studies also showed that black pepper, clove, cinnamon, and coriander exhibited antioxidant properties.

So far, a number of phenolic substances were isolated from a variety of spice sources, including phenolic acids (e.g., gallic acid, caffeic acid, etc.), flavonoids (e.g., quercetin, rutin, myricetin, luteolin, naringenin, and silybin), phenolic diterpenes, and volatile oils (Yizhong *et al*., 2005). Total antioxidant activity in 23 Iranian basil accessions was determined as Trolox equivalent antioxidant capacity (TEAC). A linear positive relationship existed between the antioxidant activity and total phenolic acids content of the tested basil accessions (Yizhong *et al*., 2005).

Spices and vegetables possess antioxidant activity that can be applied for preservation of lipids and reduce lipid peroxidation in biological systems (Wettasinghe and Shahidi, 1999). The potential antioxidant activities of some spices extracts (water and alcohol 1:1) were investigated on enzymatic lipid peroxidation. Water and alcohol extract (1:1) of commonly used spices (garlic, clove, onion, mint, cloves, cinnamon and pepper) dose dependently inhibited oxidation of fatty acids, linoleic acids in the presences of soybean lipoxygenase (Shobana and Akhilender, 2000).

Total equivalent antioxidant capacity (TEAC) and phenolic content of 26 common spice extracts from 12 botanical families were investigated. Qualitative and quantitative analyses of major phenolic in the spice extracts were systematically conducted by reversed-phase high performance liquid chromatography (RP-HPLC). Many spices contained high levels of phenolic and demonstrated high antioxidant capacity. Wide variation in TEAC values (0.55-168.7 mmol/100 g) and total phenolic content (0.04-14.38 g of gallic acid equivalent/100 g) was observed **(**Ali *et al*., 2015). Extracts of aromatic herbs, spices, and medicinal plants are employed in food processing to impart flavor and other functional properties. The extracts of some of these plants possess antioxidant, bactericidal, and anticancer properties. Although synthetic antioxidants are effective, as is the case of Butyl Hydroxy Anisole (BHA) and Butylhydroxytoluene (BHT), there are some restraints to their use because of the evidence that they may be harmful to human health (Yizhong *et al*., 2005). For this reason, it is important to consider naturally occurring antioxidants, not only to prevent food degradation but also to formulate functional mixtures for use by the pharmaceutical and cosmetic industries (Leal *et al*., 2003).

Phenolic metabolites in these plant materials are closely associated with their antioxidant activity (Yizhong *et al.,* 2005). The antioxidant effect of phenolic metabolites is mainly due to their redox properties and is the result of various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity. They are also known to play an important role in stabilizing lipid peroxidation and to inhibit various types of oxidizing enzyme. These multiple potential mechanisms of antioxidant action make the diverse group of phenolic metabolites an interesting target in the search for health-beneficial photochemical and also offer a possibility to use phenolic metabolites or extracts rich in them in lipid-rich foods to extend shelf life. The presence of antioxidative and antimicrobial phenolic constituents in many spices gives food-preserving properties as cited in Krishnaswamy, 2008.

The extracts of garlic (*Allium sativum*), and pepper (*Capsicum fructensces*) singly or combinedly consumed and dietary consumption of these spices help to neutralize free radicals in the body and may provide benefits for patients with hypercholesterolemia-induced oxidative stress (Otunola *et al*., 2014). The free radical scavenging activity of black pepper (*Piper nigrum* L.) extract increased in a concentration dependent manner and one of the fractions inhibited 55.68} 4.48% nitric oxide radicals generated, whereas curcumin in the same concentration inhibited 84.27} 4.12%. Moreover, black pepper extract scavenged the superoxide radical generated by the xanthine/xanthine oxidase system suggesting that black pepper could be a potential source of natural antioxidant (Singh *et al*., 2008 as cited in Geemon and Mariwala, 2012)

Stabilization of meat lipids with ground spices of clove, oregano, rosemary, sage and thyme tested in comminuted pork between 200– 2000ppm indicated inhibition of 2- Thiobarbituric acid reactive substances (TBARS) by 12–96.0% and reported that wise and rosemary powders were most effective increasing the shelf-life of meat products (Shahidi *et al*., 2007). Curcumins possess excellent antioxidant properties, potent in preventing lipid peroxidation than β-tocopherols. Turmeric was also reported to inhibit oxidative rancidity in salted, cooked fish and is said to be more potent than garlic and onion **(**Geemon and Mariwala, 2012**).**

2.4. Phenolic Content and Biological activity of Clove

Clove (*Syzgium aromaticum* is a branched rhizome that has a characteristic aromatic and pungent flavor belonging to the Zingiberaceae family. It has been cultivated since ancient times for cooking and medicinal preparations (Leal *et al.,* 2003). *Syzygium aromaticum* is known to be a traditional medicinal plant used as an expectorant, anti-emetic, stimulant, anti-flatulent and for treatment of dyspepsia. It is also used as an anodyne and antiseptic in dentistry (Raina *et al*., 2001). Clove extracts are rich in Polyphenolic that exhibit antioxidant activity; researchers have demonstrated that other substances present in clove extracts inhibit the growth of Mycobacterium and *Mycobacterium tuberculosis* (Leal *et al*., 2003).

Clove represents one of the major vegetal sources of phenolic metabolites as flavonoids, hydroxybenzoic acids, hydroxycinamic acids and hydroxiphenyl propens. Eugenol concentration ranging from 9381.70 to 14 650.00 mg per 100 g fresh plant materials was reported (Neveu *et al*., 2010). The major constituents of the clove essential oils are eugenol, ß-caryophyllene, α-humulene and humulene epoxide. These constituents are known to possess anti-bacterial, anti-fungal and anti-carcinogenic properties. According to these various biological activities, clove oils find uses in toothpaste, mouthwashes, soaps and other cosmetic items (Raina *et al*., 2001). Varying concentration of phenolic acids has been determined in clove extracts. Gallic acid of 783.50 mg/100 g fresh weight and gallic acid derivates as hydrolysable tannins of 2375.8 mg/100 g was reported. Other phenolic acids identified from clove extracts are the caffeic, ferulic, elagic and salicylic acids. Flavonoids as kaempferol, quercetin and its derivates (glycosylated) are also found in clove in lower concentrations (Shan *et al*., 2005).

Antioxidant activity of aqueous extracts of clove has been tested by different *in vitro* methods as 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2'-azino (3-ethylbenzothiazoline-6-sulphonic acid) (EBTS), oxygen radical absorbance capacity, ferric reducing antioxidant, radical absorbance capacity, ferric reducing antioxidant power, xanthine oxidase and 2-deoxiguanosine (Dudonne *et al*., 2009). Methanol and aqueous extracts of clove and lavender at concentrations of 20, 40 and 60 µg/mL showed inhibitions up to 95% when tested as superoxide radical capture and scavenging of the DPPH radical (Gülçina *et al.,* 2004).

2.5. Phenolics Content and Biological activity of Fennel

The *Foeniculum vulgare*, known as fennel, has a long history of herbal uses as both food and medicine (Abebie Beyazen *et al*., 2017a and b; Yaldiz and Camlica, 2019). A compiled review article indicates fennel efficacy in several *in vitro* and *in vivo* pharmacological properties such as anti-microbial, anti-viral, anti-inflammatory, anti-mutagenic, anti-pyretic, anti-spasmodic, anti-thrombotic, apoptotic, cardiovascular, chemo modulatory, anti-tumor, hepatoprotective, hypoglycemic, hypolipidemic, and memory enhancing property related to digestive, endocrine, reproductive, and respiratory systems. In general, it has been used for more than forty types of disorders (Shamkant *et al*., 2014).

Previous study revealed that anti-oxidant and anti-microbial activities of the crude extract of *Foeniculum vulgare* extracted by different solvents indicating a high potential to be used as natural antioxidants in food preservation as well as for preventing oxidative stress mediated human disorders (Abebie *et al*., 2017 A and B; Yaldiz and Camlica, 2019). Aqueous: methanol (20:80, v/v) extract of leaf contained the highest TPC [(24.3 ± 0.99) mg GAE/g], and TFC [(18.92 ± 0.34) µg CE/mg] and showed the highest DPPH radical scavenging activity (IC50 = 69.68 ± 2.28 µg/mL). The aqueous: methanol (20:80, v/v) extract had strongest reducing ability (0.82 ± 0.06 nm). Chloroform extract exhibited the highest total antioxidant capacity (1.94 ± 0.76 mg AAE/g) as determined by the phosphomolybdenum method. Except for total antioxidant activity, all antioxidant activities were positively correlated TPC and TFC (Abebie *et al*., 2017 A). Methanol extracts of seed contained the highest TPC [(22.93 ± 7.17) mg GAE/g], and TFC [(8.581 ± 1.22) µg CE/mg] and water extract showed the highest DPPH radical scavenging activity (IC50 = 207.94 ± 83.38 µg/mL) (Abebie *et al.,* 2017 B).

Polyphenolic studies have shown the presence of numerous valuable metabolites, such as volatile metabolites, flavonoids, phenolic metabolites, fatty acids, and amino acids (Shamkant *et al*., 2014). Investigation comprised of forty-six genotypes of fennel revealed trans-anethole (18.43–69.69%) as main component, while estragole (methyl chavicol) 0.27–29.55% was second most important component.

Petroselinic and myristic acids were determined as main fatty acids present in a range of 87.07% (Yaldiz and Camlica, 2019). Total flavonoid content of hydroalcoholic extracts is about 12.3 ± 0.18 mg/g. Flavonoids like eriodictyol-7-rutinoside, quercetin-3-rutinoside, and rosmarinic acid have been isolated from *F. vulgare*. Amongst the flavonoids present in *F. vulgare*, the most prevalent are quercetin-3 glucuronide, isoquercitrin, quercetin-3-arabinoside, kaempferol-3-glucuronide and kaempferol-3- arabinoside, and isorhamnetin glucoside (Kunzemann and Herrmann, 1977). Quercetin-3-O-galactoside, kaempferol-3-O-rutinoside, and kaempferol-3-O-glucoside have also been reported to occur in the aqueous extract of *F. vulgare* (Parejo *et al*., 2004).

Water extract of fennel fruits are rich in phenolic metabolites. Many of them have antioxidant activities and hepatoprotective properties. The phenolic metabolites present in *F. vulgare* are considered to be associated with the prevention of diseases possibly induced by oxidative stress such as cardiovascular diseases, cancer, and inflammation. These phenolic metabolites have received tremendous attention among nutritionists, food scientists, and consumers due to their role in human health. Fennel has been reported to contain hydroxyl cinnamic acid derivatives, flavonoid glycosides, and flavonoid aglycones (Parejo *et al*., 2004). The methanolic extract of fennel seeds contains rosmarinic acid, chlorogenic acids as major phenolic metabolites (14.9% and 6.8%, resp.), and quercetin and apigenin as the major flavonoids (17.1% and 12.5%, resp.). Also, the total phenolic metabolites in fennel methanol extract were higher than the flavonoid metabolites (Roby *et al*., 2013). *F. vulgare* has been reported to contain phenolic acids like 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O caffeoylquinic acid, 1,3-O-di-caffeoylquinic acid, 1,4-O-di caffeoylquinic acid, and 1,5-O-di-caffeoylquinic acid (Faudale *et al*., 2008).

3. MATERIALS AND METHODS

3.1. Plant Material Collection and Extraction

Flower of clove or kirunfud *(Syzgium aromaticum* (L.) Merr.) and seeds of *Foeniculum vulgare* (fennel) or ensilal were purchased from Bate town, East Hararghe, Ethiopia. The samples were shade dried and powdered. The powdered spices (50 gm) were extracted with 250 ml of solvents with increasing polarity such as, chloroform, ethyl acetate, methanol and water (Harborne, 1991) with three replications for both spice sample and all polarity gradient solvents. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator. All the extracts were subjected to qualitative and quantitative Polyphenolic analysis and antioxidant activity test.

3.2. Qualitative Analysis of Polyphenolics

Qualitative analysis of Polyphenolics of the extracts was performed to detect alkaloids, flavonoid and other phenols metabolites according to Harborne (1991).

Test for Alkaloids: Two ml of the aqueous extract was added 2ml of NH_4OH à 10% (pH=7). The alkaloid was extracted 2 times with 10ml chloroform. The chloroform layer was washed 2 times with 2 ml of HCH (10%). This was divided into two portions. Mayer's reagent was added to one portion and Wagner's reagent to the other in three replications for each. The formation of turbidity of white precipitate was regarded as positive for the presence of alkaloids (Harborne, 1991).

Test for flavonoid: Two methods were used to test flavonoid. Firstly: 1ml of the extract was treated with magnesium turnings and 2 drops of concentrated HCL (10%). Formation of pink or red color shows the presence of flavonoid. And secondly, 1ml of the extract was added to 1ml of ferric chloride for all the testube of three replication and brown color was evolved which confirms the presence of flavonoid (Harborne, 1991).

Test for Tannins and other phenols: One ml of the extract was treated with few ml of gelatin solution and white precipitate revealed the presence of tannins and terpenoids Harborne (1991).

3.3. Quantitative determination of Polyphenolic Metabolites

The total phenol content was determined using the method described by Singleton and Rossi (1965) and the concentration of total flavonoid was determined using the method described by Seung *et al.* (2003) with some modification for my thesis by three replications for all cuvette.

Determination of total phenolic content: Standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/mL) and stock solution of extracts (1 mg/ml, w/v) was prepared by dissolving extracts in their respective solvents. An aliquot (1 mL) of the standards and the extract solution were separately transferred to 25 mL capacity test tubes. One mL of Folin and Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added and mixed. The solution was then immediately diluted to volume (25 mL) with distilled water and mixed thoroughly. After incubation for 90 min at 23 ^{o} C, the absorbance versus prepared blank of all the three cuvette replication was read at 750 nm spectrophotometer. Based on this absorbance standard graph of which concentration on X axis and their respective absorbance on Y axis were drown. From the graph, the amount of phenol present in the unkown solution was calibrated using Y= mx+b, where Y = absorbance, and m= gradient (related with slope) and X = Concentration (unknown) as of Singleton and Rossi (1965). Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE/g extract DW).

Determination of total flavonoid: An aliquot (1 mL) of standard solutions of catechin (20, 40, 60, 80 and 100 µg/mL) and stock solution of extracts (1 mg/ml, w/v) was separately dissolved in 10 ml test tubes containing 4 ml ddH_2O. At zero time, 0.3 ml of 5% $NaNO_2$ was added to the flask and 0.3 ml of 10% $AlCl_3$ was added after 5 min. At 6 min, 2 ml of 1 M NaOH was dissolved in the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 ml of ddH_2O and thoroughly mixed. Absorbance of the mixture, pink in colour in all the three cuvette replicated for the sample and the standard was determined and taken at 510 nm versus prepared water blank (Seung *et al.,* 2003). Similar to phenolics, based on this absorbance standard graph of which concentration on X axis and their respective absorbance on Y axis were drown. From the graph, the amount of flavonoid present in the unkown solution was calibrated using Y= mx+b, where Y = absorbance, and m= gradient (related with slope) and X = Concentration (unknown) following method of Seung

*et al., (*2003). Total flavonoid content was expressed as milligram catechin equivalents (mg CE/g extract DW).

3.4. Assessment of Antioxidant Capacity

To determine antioxidant capacity of extracts of the spices established assays such as Diphenyl Picryl Hydrazyl (DPPH), Nitric Oxide, Hydrogen peroxide scavenging and reducing power were used.

3.4.1. Scavenging Capacity towards DPPH Stable Radical

The DPPH free radical scavenging activity of the extracts was assayed according to the method described by Abebie Beyazen *et al*. (2017 b). Different concentrations (25 to 1000 µg/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (5 mL, 0.1 mM) was prepared in methanol and added into each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference standards Butyl Hydroxy Toluene (BHT) as positive control were vortexed and left to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was then taken at 517 nm. As a negative control, methanol without the extract was used. The ability to scavenge the DPPH radical was calculated using the following (Abebie Beyazen *et al*., 2017 b) equation

$$\%\ \text{inhibition of DPPH} \cdot = \left(\frac{\text{Control}_{517\text{nm}} - \text{Sample}_{517\text{nm}}}{\text{Control}_{517\text{nm}}}\right) \times 100$$

Where, $\text{Sample}_{517\text{nm}}$ was absorbance of the sample and $\text{Control}_{517\text{nm}}$ was absorbance of positive control

DPPH radical scavenging of the extract was also measured in terms of IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that scavenges the DPPH radical by 50%. High IC_{50} value indicates less antioxidant capacity.

3.4.2. Scavenging Capacity towards Nitric Oxide Radical

The Nitric oxide (NO) scavenging capacity of the spices extract was determined by the use of Griess reaction (Garrat, 1964) with minor modification with three cuvette replication for both sample and the standard. This was done by Nitric oxide (NO) generated from sodium Nitroprusside (SNP) in aqueous solution at physiological pH.

The reaction mixture (3 mL) containing SNP (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and spice extract and BHT, standards (25-1000 µg mL^{-1}, 0.5 mL) were incubated at 25º C for 150 min. 0.5 mL of the incubated solution containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of N-1-naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand at 25º C for 30 min. The absorbance of pink colored chromophore formed during diazotization was measured at 540 nm for triplicated cuvette of both the sample & BHT of which the BHT was used for comparison. The percentage scavenging of NO^- was then calculated as (Garrat, 1964) follows:

$$\text{Inhibition of } NO^- \ (\%) \ = \left(\frac{\text{Control}_{540\text{nm}} - \text{Sample}_{540\text{nm}}}{\text{Control}_{540\text{nm}}}\right) \times 100$$

Where, $\text{Sample}_{540\text{nm}}$ is absorbance of the sample and $\text{Control}_{540\text{nm}}$ is absorbance of control.

NO^- radical scavenging of the extract was also measured in terms of IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that scavenges the NO^- by 50%.

3.4.3. Scavenging towards Hydrogen Peroxide

Scavenging of hydrogen peroxide was carried out following methods of Nabavi *et al*. (2008 and 2009). A solution of hydrogen peroxide was prepared in phosphate buffer (50 mM, pH 7.4). Extract and Ascorbic acid (standard) (25 - 1000 µg mL^{-1}, 0.3 mL) in distilled water were added to a hydrogen peroxide solution (40 mM, 0.6 ml). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard metabolites in three cuvette replication was calculated as (Nabavi *et al*., 2008 & 2009) follows:

$$\text{Scavenged } H_2O_2\ (\%) = \left(\frac{\text{Control}_{230\text{nm}} - \text{Sample}_{230\text{nm}}}{\text{Control}_{230\text{nm}}}\right) \times 100$$

Where $\text{Control}_{230\text{nm}}$ was the absorbance of the control and $\text{sample}_{230\text{nm}}$ was the absorbance in the presence of the sample of extract and standard.

3.4.4. Reducing Power

The reducing power of the extracts were performed by potassium ferricyanide reduction method (Oyaizu, 1986) to estimate the reducing power in which various concentrations of both sample extracts (0.5 mL) and standards (at 25, 50, 100, 200, 400, 800, 1000µg mL^{-1} concentrations) were separately added to a test tube containing mixture of 2.5 ml of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe_3\ (CN)_6$] (1%) solution and then vortexed. After incubation at 50°C for 20 min, 2.5 ml of TCA (10%, w/v) was added to all the tubes, and upper layer of the solution (5 mL) was mixed with deionized water (5 mL) and centrifuged at 3000 x *g* for 10 min. Thereafter, one ml of $FeCl_3$ (1%) was added to each test tube and incubated at 35°C for 10 min. The Perl's Prussian color formation was measured for the three cuvette replication at 700 nm in a UV-Vis spectrophotometer (Oyaizu, 1986). Increased absorbance of the reaction mixture indicates increasing reducing power. Ascorbic Acid (AA) was used a standard antioxidant for comparison.

3.5. Statistical Analysis of Data

All measurements were replicated three times. The data were analyzed using one-way analysis of variance (ANOVA) and the means were separated by Duncan's multiple range tests. SPSS version 20 and Microsoft excel were used for analysis operation. A probability $p < 0.05$ was considered to denote a statistically significant difference. Experimental results were expressed as means ± SD.

4. RESULT AND DISCUSSION

4.1. Qualitative Polyphenolic Analysis

The extracts of *Syzygium aromaticum* and *Foeniculum vulgare* (clove) were tested for the presence of common secondary metabolites. Polyphenolics' qualitative analysis showed the absence of alkaloid in water and methanol extracts of clove; and ethyl-acetate, chloroform and methanol extracts of fennel. Tannin was detected only in chloroform extracts of both spices. Phenols and flavonoids were found in more polar solvent extracts for both spices (Table 1). Similar results were reported by Abebie Beyazen *et al*. (2017 b) in which the tested Polyphenolics were predominantly found in water, methanol and aqueous methanol extracts of seed of *F. vulgare*. Phenolics and flavonoids are varying in polarity from less polar aglycones to very polar glycosides. Fennel has been reported to contain hydroxyl cinnamic acid derivatives, flavonoid glycosides, and flavonoid aglycones (Parejo *et al*., 2004) and clove to contain major vegetal sources of phenolic metabolites as flavonoids, hydroxybenzoic acids, hydroxycinamic acids and hydroxyphenyl propene (Neveu *et al*., 2010). In this study, the presence of tested polyphenol in a solvent of varying polarity showed that tested clove flower and fennel seeds were composed of polyphenols with different degree of polarity.

Table 1: Polyphenolic constituents of seed extracts of *F. vulgare and* flower of *S. aromaticum*

Spices	Polyphenolic	Extraction solvents				
		Hexane	Chloroform	Ethyl acetate	Methanol	Water
Clove	Alkaloids	–	+	+	–	+
	Flavonoid	+	+	+++	++	+++
	Phenols	+	+	+++	+	+
	Tannins	–	+	–	–	–
Fennel	Alkaloids	+	–	+	–	++
	Flavonoid	+	+	+++	++	+
	Phenols	+	+	+	+++	+
	Tannins	–	+	–	–	–

(-): Negative test (absence of precipitation), (+): Weak positive test (a slight presence of Polyphenolic). (+ +): Positive test (if the reactive and clearly observed Polyphenolic). (+ + +): Test strongly positive (highly precipitate or flocculation heavy).

4.2. Total Phenolic and Flavonoid Content

The level of phenolic and flavonoid metabolites in chloroform, ethyl acetate, methanol and water extracts of flower of *Syzygium aromaticum* and seeds of *Foeniculum vulgare* were presented in Table 2. From the table, amounts of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) extracted from the spices in the solvents were in the ranges of 6.23 ± 1.09 to 19.11 ± 2.76 mg GAE/g extract DW, and from 5.63 ± 1.08 to16.03 ± 1.86 mg QE/g, respectively. The aqueous extracts of both spices showed significantly the highest TPC ($p < 0.05$). Methanolic fennel and aqueous clove extracts showed significantly higher TFC ($p < 0.05$). Opposed to present study, the highest TPC of 22.93 ± 7.17 mg GAE/g was found in methanol seed extracts of fennel from Ethiopia (Abebie *et al*., 2017b). Similarly, aqueous: methanol (20:80, v/v) extract of leaf of fennel was found to contain the highest TPC (24.3 ± 0.99) mg GAE/g, and TFC (18.92 ± 0.34) µg QE/mg as compared to other solvent tested (Abebie *et al*., 2017a). Compared to present study results, much higher values of TPC were reported in some previous studies. Stoilova *et al*. (2007) reported TPC of 871 mg GAE/g in clove extract. Turgay and Esen (2015) reported 560 mg GAE/g of TPC investigating methanolic clove extract from Bulgaria and Waqas (2016) reported 54.56 ± 0.9 mg GAE/g of TPC investigating methanolic clove bud extract from Pakistan. For TPC, nearly similar TFC of hydroalcoholic extracts of seed of fennel (12.3 ± 0.18 mg/g) was reported (Parejo *et al*., 2004) and much less TFC of 3.64±0.19 mg QE/g in methanolic clove bud extract from Pakistan was reported (Waqas, 2016). Yaldiz and Camlica (2019) reported a wide range of TPC (0.27- 40.48 mg GAE/g) in fruit ethanolic extracts of 43 fennel fruit genotypes.

Fennel seed ethyl acetate extract showed significantly higher TFC than that of clove flower ethyl acetate extract ($p < 0.05$). For both spices, aqueous extract was the richest source of TPC and TFC as compared to other solvent extracts and decreased in the order of water > methanol > Ethyl acetate > chloroform extracts (Table 2). In present study, the spices were found the richest in TPC for all solvents used than the TFC. Roby *et al*. (2013) reported a similar study result that the total phenolic metabolites in fennel methanol extract were higher than the flavonoid metabolites. Studies have shown that increasing levels and diversity of flavonoids in food item could decrease certain human diseases (Parejo *et al*., 2004; Faudale *et al*., 2008; Shamkant *et al*., 2014; Yaldiz and Camlica, 2019)

The variation in content of TPC and TFC among studies may be attributed to what Jaffery (2003) stated in his study. The composition and quantity of the phenolic contents depend on different intrinsic and extrinsic factors, including plant genetics and cultivars, soil and growing condition, maturity state and harvest condition. According to Galardo *et al*. (2006), extraction process also has a significant effect on the composition and properties of the final extract. In addition to these, the total content of flavonoids and phenolics are influenced by the interaction between varieties and parts of plants used (Galardo *et al*., 2006).

Table 2: Total phenolic and flavonoid contents of seed extracts of *F. vulgare and* flower of *S. aromaticum*

Spice and extraction solvent	**TPC (mg GAE/g DW)**	**TFC (mg CE/g DW)**
Chloroform clove extract	6.23 ± 1.09^{a}	5.63 ± 1.08^{a}
Chloroform fennel extract	6.79 ± 0.55^{a}	2.90 ± 1.29^{a}
Ethyl acetate clove extract	7.98 ± 1.25^{ab}	5.83 ± 1.17^{a}
Ethyl acetate fennel extract	8.09 ± 1.44^{ab}	11.99 ± 2.12^{b}
Methanol clove extract	10.74 ± 2.00^{b}	13.25 ± 1.95^{bc}
Methanol fennel extract	9.39 ± 1.62^{ab}	16.03 ± 1.86^{c}
Aqueous clove extract	19.11 ± 2.76^{c}	15.32 ± 1.53^{c}
Aqueous fennel extract	17.35 ± 2.12^{c}	14.36 ± 2.51^{bc}

The values are Mean ± Standard deviation (n=3). Superscript letters compare means in column. Means with similar letters show no significant difference, whereas means with different letters show significant difference at $P<0.05$; DW – Dry weight

4.3. Antioxidant Activity of the Extracts

Antioxidant activity should not be concluded based on a single antioxidant test model. Antioxidant test models vary in different respects and are difficult to compare fully one antioxidant activity test method to other methods (Alam *et al*., 2012). In this study, antioxidant activity of different solvent extract of *Syzygium aromaticum* flower and that of *Foeniculum vulgare* seed was assessed by four

different methods including DPPH radical quenching, nitric oxide radical scavenging, hydrogen peroxide scavenging and reducing power.

4.3.1. Scavenging Capacity towards DPPH Stable Radical

DPPH method is based on hydrogen atom transfer and electron transfer. When a solution of DPPH is mixed with antioxidant that can donate a hydrogen atom, DPPH is reduced and loses violet color (Alam *et al*., 2012). Figure 1 shows the dose response graph of DPPH radical scavenging activity of *Foeniculum vulgare* seed of chloroform, ethyl acetate, methanol and water extracts compared with BHT. From the figure, as the concentration of extracts increased, the percent inhibition of DPPH radical also increased. Ethyl acetate extracts showed the highest DPPH scavenging at all concentration compared to the standard BHT and other extracts and these scavenging percentages were 42.88 ±1.73, 46.49 ± 1.01, 57.34 ± 2.21, 59.97 ± 2.11, 70.70 ± 1.43, 90.52± 0.56 and 94.78 ± 0.74 at 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively. According to Abebie *et al*. (2017) at concentration of 1000 µg/mL, scavenging activity of *Foeniculum vulgare* seed extracts of water, methanol and chloroform were 91.87 ± 0.043%, 80.01 ± 2.14%, 45.79 ± 2.03%, respectively. In present study, the counterpart solvents and concentrations had 79.63 ± 0.92%, 73.69 ± 1.16% and 71.51± 1.17%.

Figure 2 shows the dose response graph of DPPH radical scavenging activity of the *Syzygium aromaticum* flower of chloroform, ethyl acetate, methanol and water extracts compared with BHT. From the figure, as the concentration of extracts increased, the percent inhibition of DPPH radical also increased. The highest DPPH radical scavenging was obtained from the standard BHT at all concentration. Compared to other extracts, the scavenging activity of ethyl acetate was found the highest at all concentrations. At concentration of 1000 µg/mL, scavenging activity of *Syzygium aromaticum* were 82.62% ± 4.2, 89.46 ± 1.17%, 69.76 ± 1.64%, 76.31 ± 1.03%, 93.82 ±1.46% for chloroform, ethyl acetate, methanol and aqueous extracts, respectively. Waqas (2016) reported DPPH scavenging activity of methanolic extract of clove bud from Pakistan which had 16.5±2.5%, 22.2±1.7%, 56±4.3%, 66.6±4%, 72.94±4.9% and ±78.41±1.9% µg/ml at 25, 50, 100, 150, 200, 250 µg/ml concentrations). Gülçina *et al.* (2004) reported up to 95% inhibition of the DPPH radical by ethanol and aqueous extracts of clove at concentrations of 20, 40 and 60 µg/mL.

As compared to the fennel extracts, the higher DPPH scavenging percentage showed by non-polar solvent, chloroform may be attributed to the solvent ability to extract eugenol. Eugenol is a major constituent of the clove essential oils (Raina *et al*., 2001) and it was reviewed as a potent antioxidant polyphenol allowing the donation of hydrogen atom and subsequent stabilization of the phenoxyl radical generated from stable metabolites that do not start or propagate oxidation (Cortés-Rojas *et al*.,2014). Ghadermazi *et al*. (2017) reported that essential oil extract of clove showed 94.18±2.12%, 100.00±0.08% DPPH scavenging at 100 and 200 µg/ml concentrations which are much higher counterpart values in this study.

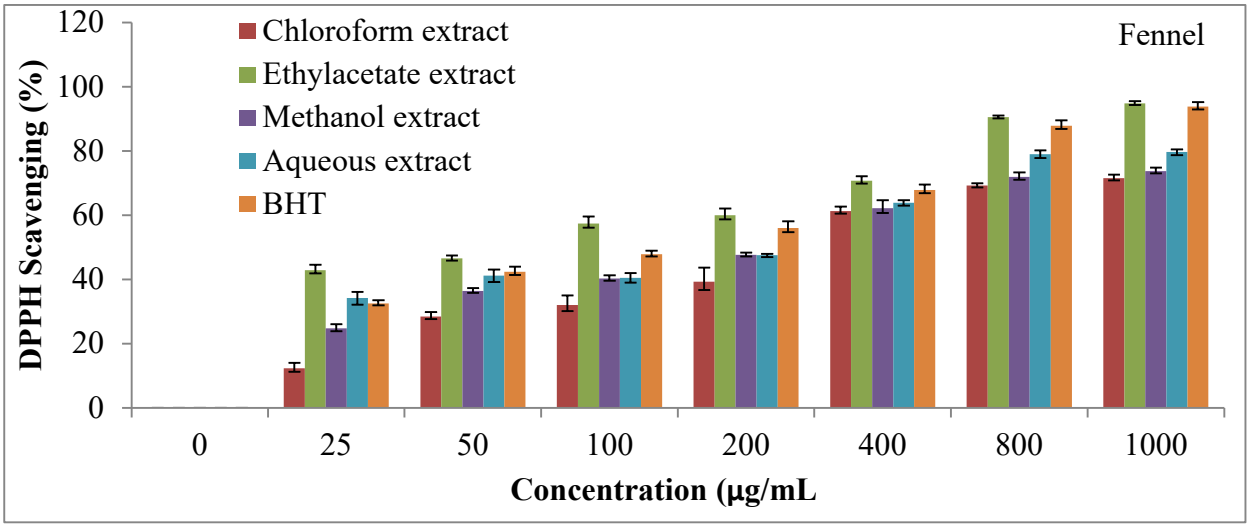

Figure 1. DPPH Radical scavenging of different solvent extracts from dried seed of *Foeniculum vulgare*

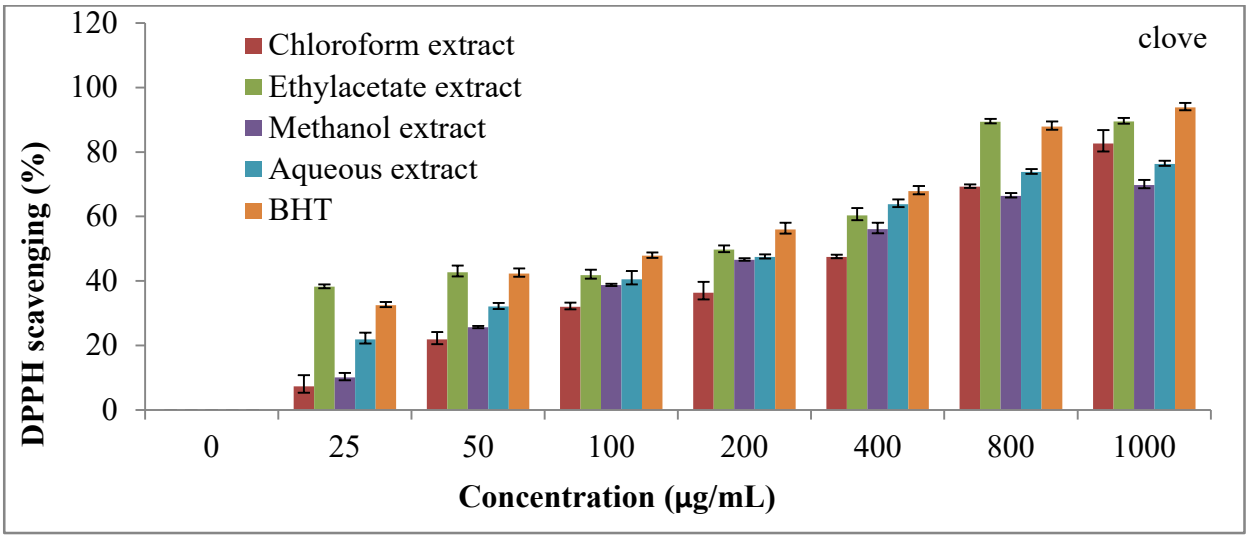

Figure 2. DPPH Radical scavenging of different solvent extracts from flower of *Syzgium aromaticum*

4.3.2. Scavenging Capacity towards Nitric Oxide Radical

Figure 3 shows the dose response graph of NO^- radical scavenging activity of *Foeniculum vulgare* seed of chloroform, ethyl acetate, methanol and water extracts compared with BHT. From the figure, it was observed as the concentration of extracts increased, the percent inhibition also increased. Chloroform extracts showed the highest NO^- radical scavenging activity at all concentration compared to the BHT and other extracts, except at 400 µg/mL. These solvent extracts scavenging percentages were 39.67±0.53, 44.50±0.68, 46.92±0.37, 58.86±0.58, 63.45±0.69, 96.08±0.50 and 97.93±0.22 at the concentrations of 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively. At concentration of 1000 µg/mL, the scavenging activity of *Foeniculum vulgare* seed were 97.93±0.22%, 80.04±0.90%, 77.31±1.11%, 83.09±0.83% and 96.86±0.83% for chloroform, ethyl acetate, methanol, water and the BHT, respectively.

Figure 4 shows the dose response graph of NO^- radical scavenging activity of *Syzygium aromatium* flower extract of chloroform, ethyl acetate, methanol and water extracts compared with BHT. From the figure, it was observed that the concentration of extracts increased, the percent inhibition also increased. Chloroform extracts showed the highest NO^- radical scavenging activity at all concentration compared to the standard BHT and other extracts, except at 25 µg/mL. These solvent extracts scavenging percentages were 32.35±1.21, 45.41±1.26, 59.31±0.82, 66.35±0.50, 80.04±0.28, 94.43±0.55 and 95.83±0.34 at the concentrations of 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively. At concentration of 1000 µg/mL, the scavenging activity of *Syzygium aromatium* flower were 95.83±0.34%, 81.48±0.80%, 63.62±0.91%, 85.43±0.26% and 81.08±0.26% for chloroform, ethyl acetate, methanol, water and the BHT, respectively.

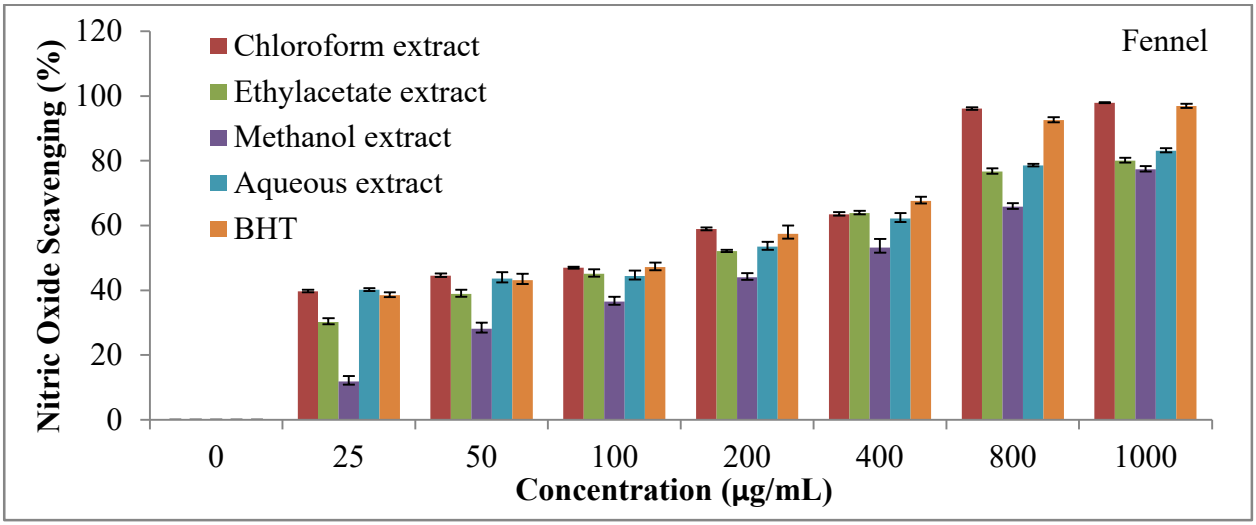

Figure 3. NO^- radical scavenging of different solvent extracts from dried seed of *Foeniculum vulgare*

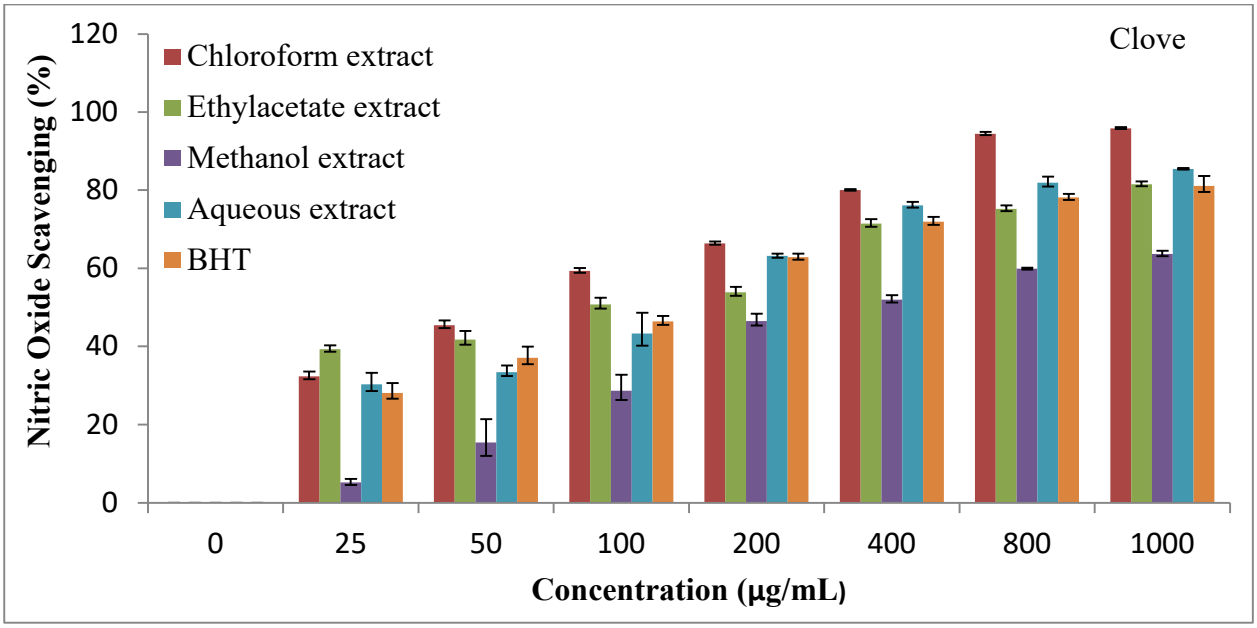

Figure 4. NO^- radical scavenging of different solvent extracts from dried seed of *Syzygium aromaticum*

4.3.3. The IC_{50} values

In this study, the parameter IC_{50} is used for the interpretation of the results from the DPPH and NO^- scavenging test method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity.

Effectiveness of antioxidant properties is inversely correlated with IC_{50} values (Abebie Beyazen *et al.,* 2017b). The IC_{50} values of all the extracts were calculated from regression curve of percentage scavenging activity against concentration of the extracts and the values were shown in Table 3. From the table, ethyl acetate fennel extract exhibited significantly the highest DPPH scavenging activity (IC_{50} =203.23±3.82) compared to both the standard and other extracts ($p < 0.05$) and methanol clove extract exhibited the lowest DPPH scavenging activity (IC_{50} = 509.48±9.88), but not significantly lower than that of chloroform fennel and clove scavenging activity ($p > 0.05$). Abebie *et al.* (2017b) reported water extract of seed fennel from Ethiopia had the highest DPPH radical scavenging activity (IC_{50} = 207.94 ± 83.38 µg/mL) opposing the result of this study. Similar to present study, these researchers found chloroform extract exhibited the weakest scavenging potency (IC_{50} > 1000 µg/mL). Lower than present study IC_{50} value for methanol extract of wild fennel (31 µg/mL) and cultivated fennel seeds (83 µg/mL) reported by Conforti *et al.* (2006). Similary, Turgay and Esen (2015) reported methanolic clove extract of Bulgaria origin that had DPPH scavenging activity (IC_{50} = 136.6 mg/mL). Waqas (2016) obtained DPPH scavenging (IC_{50}= 121.81+0.9 µg/ml) for methanolic clove bud of Pakistan origin.

Chloroform clove extract showed significantly highest NO^- radical scavenging (IC_{50} =192.94±1.9) and methanol clove extract showed significantly the lowest NO^- radical scavenging (IC_{50} = 605.84±5.36) (Table 3). For clove solvent extracts, the order in potency of NO^- radical scavenging was chloroform (IC_{50}=192.94±1.9) > ethyl acetate (IC_{50} =290.37±3.20) > water (IC_{50} = 296.16±6.72) > methanol (IC_{50} = 605.84±5.36).

DPPH and NO^- radical scavenging abilities of some extracts were less than that of BHT used as standard as it is noted from IC_{50} values. The study showed that at higher extracts concentration, all the extracts have a best the proton donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Table 3. IC_{50} values of radical scavenging activities of extract of *Syzygium aromatium* and *Foeniculum vulgare*

Solvent extracts of the spices	IC_{50} (µg/mL)	
	DPPH scavenging	NO^- scavenging
Chloroform *Syzygium aromatium* extract	502.40±10.17^f	192.94±1.9^a
Chloroform *Foeniculum vulgare* extract	496.38±8.06^f	241.88±44^b
Ethyl acetate *Syzygium aromatium* extract	303.56±13.14^c	290.37±3.20^c
Ethyl acetate *Foeniculum vulgare* extract	203.23±3.82^a	349.98±4.83^e
Methanol *Syzygium aromatium* extract	509.48±9.88^f	605.84±5.36^g
Methanol *Foeniculum vulgare* extract	419.12±15.17^e	489.94±5.40^f
Aqueous *Syzygium aromatium* extract	413.14±10.52^e	296.16±6.72^c
Aqueous *Foeniculum vulgare* extract	351.85±23.18^d	310.36±10.22^d
BHT	256.38±25.41^b	247.64±12.89^b

The values are Mean ± Standard deviation (n=3). Superscript letters compare means in column. Means with similar letters show no significant difference, whereas means with different letters show significant difference at $P<0.05$.

4.3.4. Scavenging Capacity towards Hydrogen Peroxide

Figure 5 shows the dose response graph of H_2O_2 free radical scavenging activity of *Foeniculum vulgare* seed of chloroform, ethyl acetate, methanol and water extract compared with Ascorbic Acid (AA). From the figure it was observed that, as the concentration of extracts increased, the percent inhibition also increased. All solvent extracts showed the higher H_2O_2 free radical scavenging activity at all concentration compared to the AA, chloroform at 1000 µg/mL. Among the spice extracts, the potent H_2O_2 free radical scavenger was methanolic extract at all concentration, except 400 µg/mL. This solvent extract showed 50.77±1.85, 54.79±1.57, 60.00±1.85, 67.26±0.39, 69.66±0.30, 92.74±2.31 and 96.24±0.65 scavenging percentages at 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively. At concentration of 1000 µg/mL, the scavenging activity of *Foeniculum vulgare* seed were 40.26±1.60%, 92.99±0.39%, 96.24±0.65%, 72.14±0.65% and 64.87±0.26% for chloroform, ethyl acetate, methanol, water and the AA, respectively.

Figure 6 shows the dose response graph of H_2O_2 free radical scavenging activity of *Syzygium aromatium* flower extract of chloroform, ethyl acetate, methanol and water extracts compared with AA. From the figure, as the concentration of extracts increased, the percent inhibition also increased. All solvent extracts showed the higher H_2O_2 free radical scavenging activity at all concentration compared to the AA. Among the spice extracts, the potent H_2O_2 free radical scavenger was methanolic extract at all concentration, except 25 µg/mL.

This solvent extract showed 37.35±2.33, 45.47±0.90, 52.99±2.3, 61.62±0.53, 68.12±0.30, 89.74±0.44 and 92.82±0.26 scavenging percentages at 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively. At concentration of 1000 µg/mL, the scavenging activity of *Syzygium aromatium* flower were 64.19±0.78%, 86.67±1.68%, 92.82±0.26%, 67.86±0.39% and 64.87±0.26% for chloroform, ethyl acetate, methanol, water and the AA, respectively. In previous study, Waqas (2016) reported H_2O_2 free radical scavenging by clove bud methanolic extract from Pakistan origin which had 18.8 ± 0.45%, 25.82±0.8%, 35.7±1.1%, 48±0.12%, 59.2±1.5% and 71±0.57% µg/ml at 25, 50, 100, 150, 200, 250 µg/ml concentrations, respectively. Ghadermazi *et al*. (2017) reported much lower H_2O_2 free radical scavenging by essential oil extract of clove (0.95±0.03%, 1.10±0.08%, 2.04±0.11%, 2.68±0.02%, 2.8±0.04% and 2.92±0.03% at concentration range 100-1000 µg/ml, respectively).

The H_2O_2 free radical scavenging property of *Foeniculum vulgare* seed and *Syzygium aromatium* flower solvent extracts attributed to presence of structural molecules that act as reducing agent. Waqas (2016) stated that phenolic scavenge hydrogen peroxide by donating their electron to free radical and convert it into reduced form. As hydrogen peroxide is free radical which is cytotoxic and cause damage to the cell, the scavenging activity of *Foeniculum vulgare* seed and *Syzygium aromatium* flower is very crucial to prevent the cell from damage.

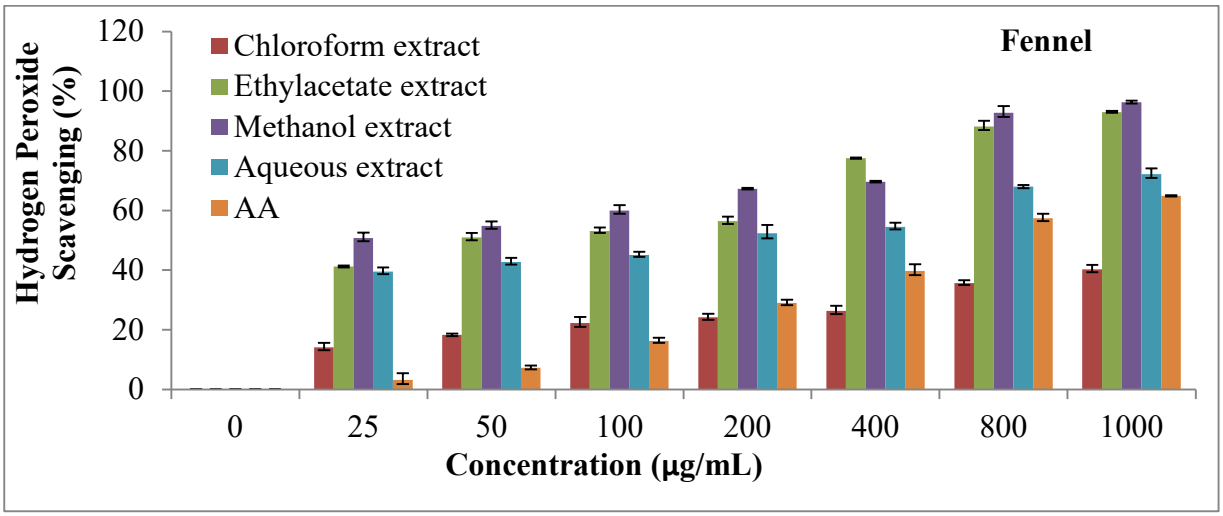

Figure 5. H_2O_2 scavenging of different solvent extracts from dried seed of *Foeniculum vulgare*

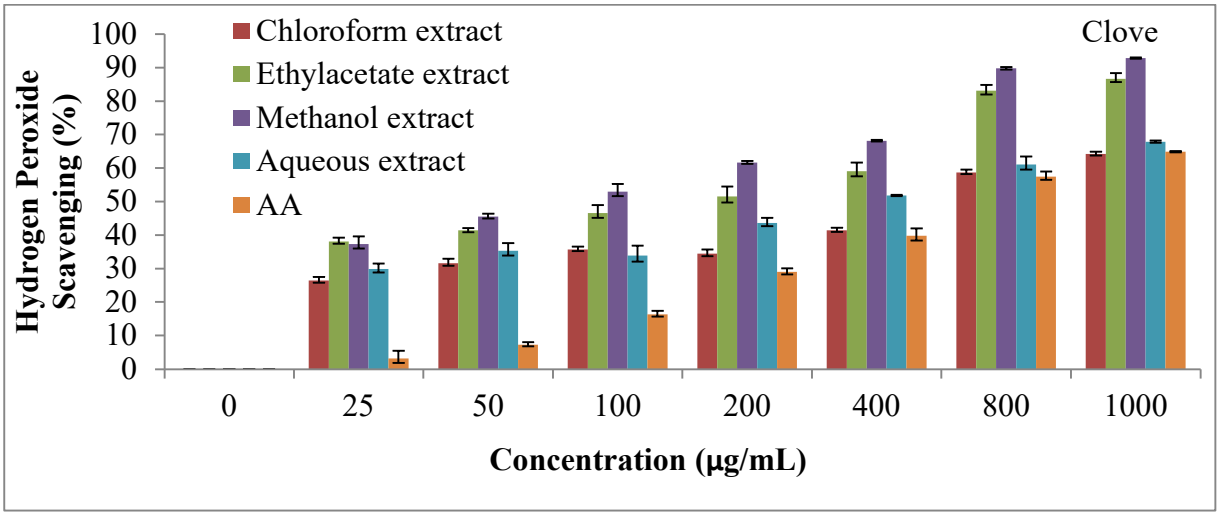

Figure 6. H_2O_2 scavenging of different solvent extracts from dried seed of *Syzygium aromaticum*

4.3.5. Reducing Power

Figure 7 shows the dose response graph of reducing power of *Foeniculum vulgare* seed of chloroform, ethyl acetate, methanol and water extracts compared with BHT. From the figure, as the concentration of extracts increased, the absorption at 700 nm (reducing power) also increased. All solvent extracts showed the lower reducing power at all concentration compared to the standard (AA). Among the extract, the best reducing power was showed by ethyl acetate extracts. This solvent extracts reducing power were 0.12±0.00, 0.14±0.01, 0.17±0.01, 0.19±0.01, 0.22±0.00, 0.32±0.02, and 0.34±0.01 at the concentrations of 25, 50, 100, 200, 400, 800 and 1000 µg/mL respectively. At concentration of 1000 µg/mL, the reducing power were 0.27±0.04, 0.34±0.01, 0.30±0.01, 0.27±0.02 and 1.20±0.06 for chloroform, ethyl acetate, methanol, water and the BHT, respectively. At 1000 µg/mL of extracts, the reducing power of *Foeniculum vulgare* seed extracts with values 0.598 ± 0.02, 0.504 ± 0.02 and 0.47 ± 0.03 for methanol water and chloroform extracts, respectively, were reported (Abebie *et al*., 2017b). These reducing powers were greater than that of the present study counterparts. The same researchers reported reducing power of *Foeniculum vulgare* leaf extracts with values 0.349 ± 0.114, 0.338 ± 0.039 and 0.237 ± 0.053 for chloroform, methanol and water extracts, respectively at 1000 µg/mL (Abebie *et al*., 2017a).

Figure 8 shows the dose response graph of reducing power of *Syzygium aromaticum* flower of chloroform, ethyl acetate, methanol and water extracts compared with BHT. From the figure, as the concentration of extracts increased, the absorption at 700 nm (reducing power) also increased. All

solvent extracts showed the lower reducing power at all concentration compared to the standard (AA). Among the extract, the best reducing power was showed by methanolic extract. This solvent extracts reducing power were 0.11±0.02, 0.13±0.02, 0.15±0.01, 0.20±0.03, 0.26±0.04, 0.34±0.04 and 0.36±0.05 at 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively. At concentration of 1000 µg/mL, the reducing power were 0.29±0.00, 0.19±0.01, 0.36±0.05, 0.32±0.01 and 1.20±0.01 for chloroform, ethyl acetate, methanol, water and the BHT respectively.

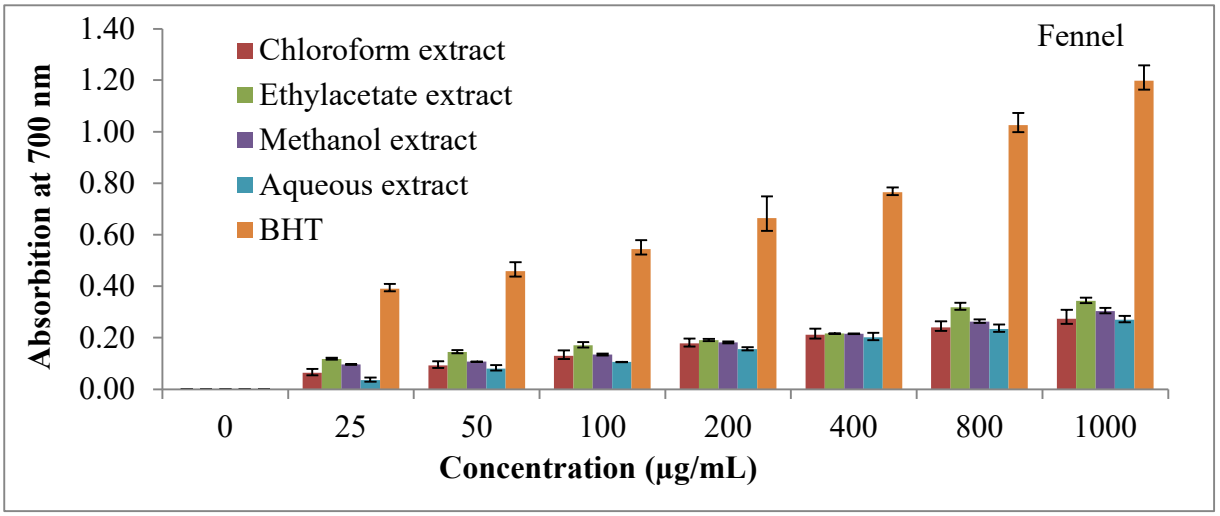

Figure 7. Reducing power of different solvent extracts from dried seed of *Foeniculum vulgare*

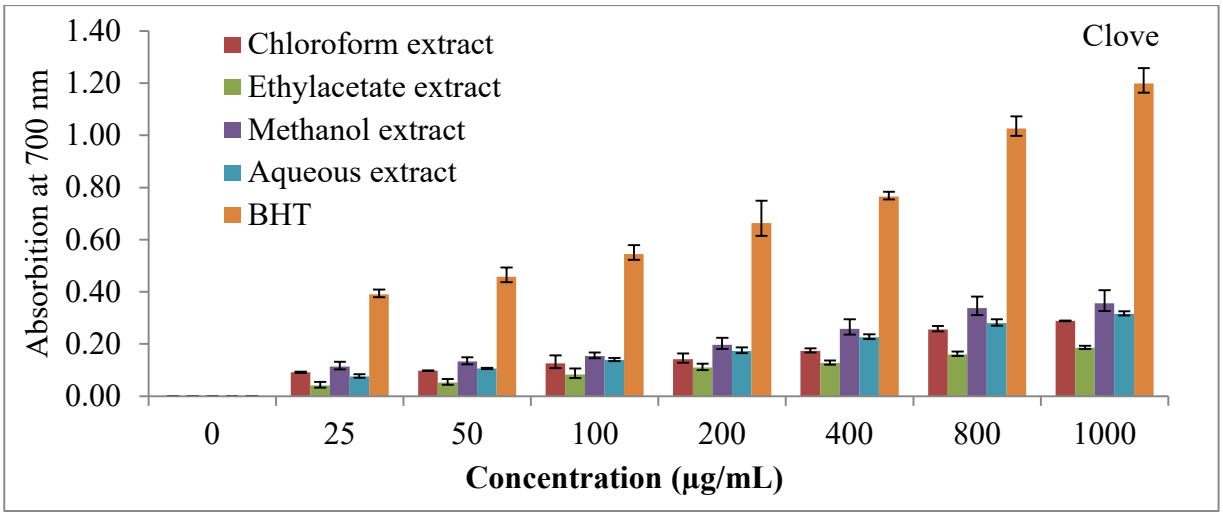

Figure 8. Reducing power of different solvent extracts from dried seed of *Syzygium aromaticum*

5. SUMMARY, CONCLUSION AND RECOMMEMNDATION

5.1. Summary

Spices have been investigated for their antioxidant properties for many years. Fennel (*Foeniculum vulgare*) and Clove (*Syzygium aromaticum*) extracts are rich in Polyphenolic that exhibit antioxidant activity. The general objectives of this study were to analyse polyphenols and antioxidant activity of Ethiopian Clove (*Syzygium aromaticum* (L.) Merr**.**) and Fennel (*Foeniculum vulgare* Mill.) using different solvent extracts and the specific objectives were to identify the presence of main polyphenols in the extracts, determine the quantity of total polyphenol and flavonoid, and evaluate antioxidant properties of the extracts.

To achieve these specific seed of fennel and flower of clove were purchased, dried and powered. The extraction was done using chloroform, ethyl acetate, methanol and water. Qualitative analysis of the obtained extracts was performed using standard procedure for alkaloids, flavonoid, Tannins and other phenols. The concentration of total phenols and flavonoid of the extracts was determined using spectrophotometer technique. Antioxidant capacity of extracts of the spices was determined using DPPH, Nitric Oxide, Hydrogen peroxide scavenging and reducing power.

Polyphenolic qualitative analysis showed the absence of alkaloid in water and methanol extracts of clove and ethyl chloroform and methanol extracts of fennel. Tannin was detected only in chloroform extracts of both spices. Phenols and flavonoids were predominantly presented in more polar solvent extracts of fennel and clove.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) extracted from the spices in the solvents were in the ranges 6.23 ± 1.09 to 19.11 ± 2.76 mg GAE/g and from 5.63 ± 1.08 to16.03 ± 1.86 mg QE/g, respectively. The aqueous extracts of both spices showed significantly the highest TPC. Methanolic fennel and aqueous clove extracts showed significantly higher TFC.

Antioxidant activity tests showed that as the concentration of spices extracts increased, the percent inhibition of DPPH, NO^-, H_2O_2 radicals and reducing power also increased. Ethyl acetate fennel extracts showed the highest DPPH scavenging at all concentration compared to the standard (BHT) and other extracts, and that of cove also showed the highest compared to other solvent extract at all concentrations, except BHT.

Chloroform fennel extracts showed the highest NO^- radical scavenging activity at all concentration compared to the standard (BHT) and other extracts, except at 400 µg/mL, and chloroform clove extracts showed the highest NO^- radical scavenging activity at all concentration compared to the standard (BHT) and other extracts, except at 25 µg/mL. Among the fennel and clove spice solvent extracts, the potent H_2O_2 free radical scavenger was methanolic extract at all concentration, except at 400 µg/mL for fennel and 25 µg/mL for clove. For fennel, the best reducing power was showed by ethyl acetate extracts having 0.12±0.00, 0.14±0.01, 0.17±0.01, 0.19±0.01, 0.22±0.00, 0.32±0.02 and 0.34±0.01 absorptions at 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively, and for clove, the best reducing power was showed by methanolic extract having 0.11±0.02, 0.13±0.02, 0.15±0.01, 0.20±0.03, 0.26±0.04, 0.34±0.04 and 0.36±0.05 absorptions at 25, 50, 100, 200, 400, 800 and 1000 µg/mL, respectively.

5.2. Conclusion

Generally, the study showed more polar clove and fennel extracts was rich in total flavonoid and other phenols. The radical scavenging abilities of the extracts were less than those of ascorbic acid and BHT at the same concentration, the study showed that the extracts have the proton – donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Free radicals scavenging of the both spices were found the highest for the same solvent, but the highest reducing of the fennel and clove was observed for different solvent. The results indicated the extracts has proton donating ability which could serve as free radical inhibitors or scavengers as showed higher values than tested standard in most cases. And Among the extract, the best reducing power were showed by methanolic extract. At the rest, both spices and their respective extraction solvent showed less reducing power comparing to the standard.

5.3. Recommendation

Based on the scope, the results of the study and others researcher finding on related articles and spices, the following recommendations are forwarded

- Extraction process of the spices should be carefully undergo not to affect the composition and properties of the final extract
- Since increasing levels and diversity of flavonoids in food item could decrease certain human disease, the level and diversity of polyphenolic contents should be tested along with targeted human disease.
- Antioxidant activity of the spices should be tested with other Antioxidant activity testing methods
- Since the concentration and percent inhibition toward DPPH, NO^-, H_2O_2 radicals has checked to have direct proportion, the dos related further activity should be undertaken for oxidative stress human disease remedies.
- In *vivo* antioxidant activity of the spices should be carried out.

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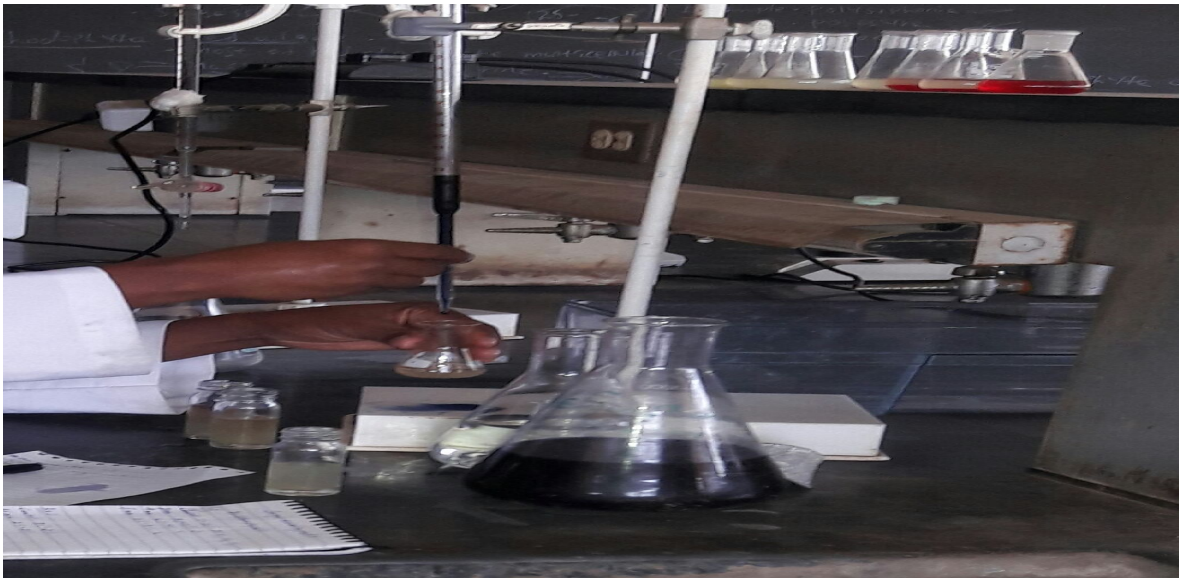
7. APPENDICES

7.1.Sample of diagraph on DPPHA Dilution into six different concentration with methanolic solvents (25 to 1000 µg/mL) in analysing antioxidant activity



7.2.Sample of diagraph of standard Gallic acid concentration (20 to 100 µg/mL)

7.3. DPPH oncentration gradient response by decolorization changes from purple