ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *Sclerocarya birrea* (A. Rich.) Hochst SEED AND LEAF EXTRACTS

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APPROVAL SHEET

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I dedicate this thesis to my wife Hayat Osman for providing unforgettable and valuable encouragements in my academic career while I was conducting this study.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

The author was born on April, 1988 in Jerso *Woreda* located in East Hararghe, Oromia Regional State. He attended his elementary education at Adam Caffe Primary School. He attended hishigh School education atJerso Secondary School. He also attended his preparatory School education at Gursum Secondary School.Upon successful completion of his preparatorySchool, he joined Jigjiga University in2011 and graduated on June , 2013 with B.Sc. degree in Biology. After graduation he was employed at Meyu*Woreda* as a teacher profession. He was then given the opportunity to join the Postgraduate Program at Haramaya University to pursue his M.Sc. degree in Biology in July, 2018.

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ACRONYMS AND ABBREVIATIONS

MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MHA	Muller Hinton Agar
NA	Nutrient Agar
MIC	Minimum Inhibitory Concentration
DPPH	Diphenyl picrylhydrazyl
PDA	Potato Dextrose Agar
ANOVA	Analysis of Variance
HPSA	Hydrogen Peroxide Scavenging Assay
LSD	Least Significance Difference
CRD	Complete Randomized Design

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ABSTRACT

Marula (Sclerocarya birrea (A. Rich.)Hochst.)is often referred to as the "tree of life "since its leaves, stem barks, roots, and fruits are used as food and traditional medicine. The present study is undertaken to investigate antioxidant and antimicrobial activities of the aqueous and methanolic extracts of S. birrea leaf and seed extracts. Qualitative analysis of major secondary metabolites including alkaloids, flavonoids, saponins, steroids, tannins and terpenoids of the S. birrea crude extracts were carried out using standard methods. The antimicrobial experiment was arranged as three factor experiment with extracts: leaf and seed of S. birrea; water and methanol; two bacteria: E. coli and S. aureus, and two fungi: Aspergillus versicolor, and A. niger in three replications. A complete randomized design (CRD) was used to determine the antimicrobial activities using disc diffusion and broth dilution methods. The results of the phytochemical screening of leaf aqueous extract has revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and steroids while alkaloids, saponins, flavonoids, terpenoids, tannins, and steroids were detected in methanolic leaf extract of S. birrea. The antioxidant activity of the S. birrea crude extract as measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH)method indicated the highest DPPH radical scavenging activity (39.25%), hydrogen peroxide scavenging activity (17.77%) in the aqueous seed extract. The highest concentration of the methanolic leaf extract (200mg/ml) presented the strongest antibacterial activity showing the maximum zone of inhibition (18.53mm) against S. aureus. On the other hand, the seed aqueous extract presented the weakest antibacterial activity with the lowest inhibition zone of 13.67mm against E. coli. The highest concentration of the extract revealed the highest antifungal activity (19.93mm) that was recorded for methanolic seed extract against A. versicolor. The methanolic extract from leaf presented the strongest antibacterial activity with a Minimum inhibitory concentration (MIC) of 3.13mg/ml and a corresponding Minimum Bactericidal Concentration (MBC) of 3.13mg/ml against S.aureus. The strongest antifungal activity with (MIC) of 6.25mg/ml and the corresponding Minimum Fungicidal Concentration (MFC) of 6.25mg/ml was recorded for methanolic extract of S. birreaagainst A. versicolor. Furthermore, S. aureus was the more susceptible to the antibacterial activity while A. versicolor was more susceptible to the antifungal activity of the S. birrea crude extracts.

Keywords: Antibacterial potential, Antifungal activity,Broth dilution, Disc diffusion , Free radical scavenging activity.

1. INTRODUCTION

Marula (*Sclerocarya birrea* (A. Rich.)Hochst.)is often referred to as the “tree of life” due to its ability to provide fundamental human need like food and medicine (Shackleton *et al.,* 2002). It is a member of the Anacardiaceae, mango and cashew nut family. *Sclerocarya birrea* subsp. *caffra* occurs in East and South tropical Africa as well as Madagascar (Chirwa and Akinnifesi, 2008). It is a popular African wild fruit tree distributed in Southern, Western and Eastern African countries where the leaves, stem bark, root, and fruits are used in food and traditional medicine; the fruit is rich in ascorbic acid. The fruit juice contains sesquiterpene hydrocarbon, which are terpenes found in plants that are reported to have bacteriostatic properties. The fruit contains a hard brown seed. The seed encloses a soft white kernel rich in oil and protein. The oil contains oleic, palmitic, myristic, and stearic acids; the kernel protein contains amino acids, with a predominance of glutamicacid and arginine. The extracts from different parts showed high total phenolic compounds and radical-scavenging capacities and antioxidant activities (Mariod and Abdelwahab, 2012).

Marula has the potential of an industrial fruit crop because of the high spectrum of valuable products that can be prepared from its fruits and because of its high yields. In addition, harvesting can be easily mechanised (fruits are collected from the ground); yielding period lasts for several months (Nerd and Mizrahi, 2000).Because of its high potential fruit production and uses, the marula tree has been identified as a key species to support the rural development enterprises based on the fruit, beer, oil and nuts and thus making it a species with high potential domestication value (Shone, 1979; Shackleton *et al*., 2002c).

The marula is a valued fruit tree and all parts of the fruit are edible. The juicy pulp is rich in vitamin C. It is used in jams and jellies and, on a commercial basis, to produce marula beer in Southern Africa. The seeds have a high oil content (~60%), consisting mainly of unsaturated fatty acids, and are also rich in protein and minerals. They are eaten raw or cooked (Orwa *et al.*, 2009). The wood is used for carvings and fuel, fibres from the inner bark for making rope, fruits and leaves are a source of fodder and the tree provides shade and acts as a wind break (Jøker and Erdey, 2003).

The fruit pulp/juice contains up to 2 mg of vitamin C per gram, which is four times that of orange juice (Shone, 1979). Vitamin C is an important anti-oxidant, helps protect against cancers, heart disease, stress, helps in maintaining ahealthy immune system, it aids in neutralizing pollutants, is needed for antibody production, acts to increase the absorption of nutrients (including iron) in the gut and thins the blood; it is essential for sperm production and for making the collagen protein involved in the building and health of cartilage, joints, skin and blood vessels (Anon; 2004). Taylor *et al.* (1995) stated that the high vitamin C content in the fruit as well as the oils and other nutrients in the nuts provide people, especially children, with important nutritional requirements and the fruitcan be used to make chutneys and pie fillings. The juice of its fruits is pleasantly acidic, sour-tasting and refreshing (Hall *et al*., 2000).

The seed is rich in protein and oil, magnesium, phosphorus and potassium, which make it important to an African diet. The seeds are eaten, dried or ground and added to soups, stews and vegetables, to which they are reputed to give a delicious flavour. Fresh seeds are also added to newly–boiled meat, which is then eaten immediately (Shackleton *et al.,* 2002c). The seed contain about 60% of non-drying oil, which is rich in protein. The oil from the nuts is used for cooking, for flavouring in porridge, as a skin moisturiser, medicine and insecticide. It is especially valued by the cosmetic industry due to its slow oxidizing properties (Duke, 1989; Hall *et al*., 2000).

Almost all parts of the plant, but especially the bark and leaves, are exploited for medicinal uses (Hall *et al*., 2000). The stem-bark aqueous extract was given to rats to control diabetes and the results of the study indicated that the aqueous extract of marula tree possessed hypoglycaemic activity and thus lend credence to the suggested folkloric use of the plant in the management and control of adult onset of type-2 diabetic mellitus in some African communities (Ojewole, 2003). The medicinal properties of the bark is used widely in treating dysentery and diarrhoea, rheumatism, gangrenous rectitis, insect bites, burns and a variety of other ailments due to its high antibacterial activities (Hall *et al*., 2000).

The bark is also used as an anal suppository (powder) for treating haemorrhoids. Powdered bark mixed in a drink of milk or millet water is used to reduce fevers (Hall *et al*., 2000). Bark

decoction, together with butter, is applied as an ointment for headache and pains of the eyes. Leaves, bark and roots are used externally (as a rub) for snake bite, and internally (as a beverage) for toothache. It has occasionally been used in veterinary medicine. A red dye is also obtained from the bark (Shone, 1979). Thirty-six woodland tree species with medicinal uses were recorded as having had bark harvested from one to more individuals. Decoctions, infusions or steam from boiled roots is used to treat heavy menstruation, bilharzia, coughs, weakness, sore eyes, heart pains and as an antiemetic (Shackleton *et al.,* 2002c).

Essence from the leaves provides a remedy for abscesses, spider bites and burns. The leaves are also used as a sedative (Shackleton *et al.,* 2002c).Leaves can also be used in compost-making (Sunday Sun, 2003). The leaves and leafy stems/branches make good animal feed, both for wild game and livestock especially during the winter months (Hall *et al*., 2000; Shackleton *et al.*, 2002c). One of the reasons why marula is protected is because buffalo grass, *Panicum maximum*, grows under the tree. The gum secreted by marula is used to make ink by dissolving it into water and adding soot (Marula Net Database, 2003) and fibres from the inner bark for making ropes (DFSC, 2003).

The tree is a host to the edible mopane caterpillar. Shade or shelter: *S. birrea* ssp. caffra can be used most successfully as a shade tree in the garden or park and to line streets. Boundary or barrier or support: Cuttings and truncheons strike readily and *S. birrea* ssp. caffra can be used to make a live fence (Orwa *et al.,* 2009). The larvae of mopane worm (*Imbrasia belina*) have been reported to occur on many different indigenous and exotic plant species, as well as on the marula tree. Interplanting of marula tree in mopane (*Colophosepermum mopane)* stands might alleviate the defoliation pressure of the mopane moth and would also benefit the community as they could harvest the fruits of marula for beer production and selling (Lawes *et al*., 2004). Even though *Sclerocarya birrea*is widely studied with regard to its antidiabetic, anti-inflammatory, analgesic, antiparasitic, antimicrobial, and antihypertenisve activities, no information is available for Ethiopian varieties. The *S. birrea* plant is distributed in low and midaltitude regions of East Hararghe, Ethiopia. Therefore, the present study is undertaken to investigate antioxidant and antimicrobial activities of aqueous and methanolic extracts of *S. birrea* leaf and seed extracts.

Objectives

General Objective**:**

- To assess antioxidant and antimicrobial activities of *S. birrea*leaf and seed extracts.

Specific Objectives:

- To screen the phytochemical constituents of *S. birrea* leaf and seed extracts
- To test antioxidant activities of *S. birrea* leaf and seed extracts
- To determine the antibacterial and antifungal activities of *S. birrea* leaf and seed extracts;
- To determine the minimum inhibitory concentration (MIC) of the crude extracts;
- To determine the minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) of the crude extracts.

2. LITERATURE REVIEW

2.1. Botanical Description

Marula *(Sclerocarya birrea* (A. Rich.)Hochst.)belongs to the family Anacardiaceae. Synonyms: *Commiphora subglauca* Engl.;*Poupartia caffra* (Sond.) H. Perrier; *Sclerocarya caffra* Sond.;*S. caffra* Sond. var. *dentata* Engl.; *S. caffra* Sond. var. *oblongifoliata* Engl.; *S. schwein-furthiana* Schinz Vernacular/common names: marula (English name); hameid (Arabic); didiiysa (local name in Oromo). Subspecies/Varieties: *Sclerocarya birrea* subsp. *birrea*; *Sclerocarya birrea* subsp. *caffra* (Sond.) Kokwaro; *Sclerocarya birrea* subsp. *multifoliata* (Engl.) Kokwaro (Jøker and Erdey, 2003). The tree is native to Africa where it is widely distributed between 16°N and 20°S in wooded grasslands, riverine woodlands and bush lands. It prefers well drained sandy soils and loams but is often found growing on rocky hills and is intolerant to frost. Occurs at low to medium altitudes in areas with 200-1600 mmof rainfall per year. Subsp. caffra is known to be highly salt tolerant (Jøker and Erdey, 2003).

Marula *(Sclerocarya birrea)* tree, normally about 10 m tall, on favourable sites up to 20 m. Bark grey, flaking in patches exposing the underlying light yellow tissue. Leaves alternate, compound, with 7-13 pairs of opposite leaflets plus the terminal leaflet (Palgrave, 2002). Leaflets are dark green above, much paler and bluish-green below. The leaves are crowded near the ends of branches. The species is principally dioecious with male and female flowers on different trees, but occasionally a tree can bear flowers of both sexes. Flowers in 5-8 cm long inflorescences at the end of branches. The fruit is round drupe, up to 3.5 cm in diameter, yellow at maturity. The pulp is juicy and adheres tightly to the stone. The stone is 2-3 cm long, hard, with one to four cavities, each usually containing one seed. Each cavity has an opening covered with a lid (operculum) that remains firmly attached until germination. The seeds are small and fragile, covered with a thin seed coat. 500 stones per kg has been reported (Jøker and Erdey, 2003).

The trees are deciduous, standing bare for several months during the dry season. Flowering occurs at the end of the dry season just before the leaves appear and the fruits mature at the beginning of the rainy season. The fruits abscise before they are mature. At the time of fruit

fall the fruits are still green and firm and final ripening takes place on the ground. Trees can begin to set seed as early as at the age of 5 years. When the fruits have turned yellow they are mature. At this stage they have already been abscised, so fruits are normally collected from the ground. When mature, the seeds have a high moisture content, up to 30% and to avoid fermentation of the pulp the fruits must be brought to the processing site as soon as possible (Jøker and Erdey, 2003).

2.2. Biochemical Composition and Proximate Analysis

The nutrient composition of*Sclerocarya birrea*(A. Rich.) Hochstfruit revealed that it is rich in ascorbic acid and the fruit juice contains sesquiterpene hydrocarbons (including caryophyllene, α-humulene, and copaene). The fruit kernels contain high amount of oil and protein. The oil-rich seeds contain oleic, myristic, and stearic fatty acids and different types of amino acids, with a predominance of glutamic acid and arginine. The bark yields 3.5–20.5% tannin, 10.7% tanning matter, and traces of alkaloids (Watt and Breyer-Brandwijk, 1962). Tanninsand flavonoids are present in leaves but no alkaloids, steroids, or triterpenoids have been detected (Gueye, 1973).

The fruit is rich in ascorbic acid and juice extracts yield 33 sesquiterpene hydrocarbons (Pretorius *et al.*, 1985). The fruit contains two to three edible kernels, which contain 53.0%, 28.0%, and 8.0% of oil, protein, and carbohydrates, respectively. The gum is rich in tannin(0.4%). Tannins and flavonoids are present in leaves but no alkaloids, steroids, or triterpenoids have been detected (Gueye, 1973).

2.2.1.Protein content Marula (*Sclerocarya birrea)*

The protein contains a good level of sulfur-containing amino acids (methionine and cysteine), when compared to that of four different food materials. The invitroprotein digestibility of *Sclerocarya birrea* was almost similar to that of soy bean protein concentrate and less than that of lupine, where 79% of the *Sclerocarya birrea* seed protein was found digestible by pancreatic enzyme, which was similar to 79% of soybean concentrate and less than 83.2% of lupine (Mariod *et al.*, 2005). With a chemical score of 33.0%, based on the essential amino

acids pattern requirements for children, the limiting amino acid in *Sclerocarya* protein is lysine (Mariod *et al.,* 2005).

Glew *et al.* (2004) reported a protein content of 36.4% of dry weight; however, the protein fraction contained relatively low proportions of leucine, phenylalanine, lysine, and threonine. Because of the widespread occurrence, potentially high fruit production, and use of *Sclerocarya birrea*, products have been produced from seed kernels by adding them to Halva confectionary (a confectionary made of sesame paste and mixed sugars) and biscuits. In the case of Halva confectionary, 10% unroasted, blanched, and roasted kernels were added, whereas in biscuits *Sclerocarya* seed oil instead of hydrogenated oils was used. The results showed a statistically significant difference ($P \leq 0.05$) in texture, flavor, and overall preference between the Halva developed products and those produced using Sclerocarya seed oil Biscuits processed by using the seed oil instead of hydrogenated oils were found to be significantly ($P \leq 0.05$) less acceptable using sensory scores (1–9) than the conventional biscuits. *Sclerocarya birrea* oil was used in blending cosmetics and biodiesel products with for its high stability (Mariod *et al.,* 2005).

Oxidative stability is an important parameter for evaluating the quality of oils and fats, as it gives a good estimation of their susceptibility to oxidative deterioration, themain causeof their alteration (Mateos *et al.,* 2005). The oxidative stability of *Sclerocarya birrea* oil, as measured by the Rancimat test at 120 ◦C, was 43 hours. This high oxidative stability may be due to a high percentage of monounsaturated fatty acids in addition to other minor bioactive components such as sterols and phenolics (Mariod *et al.,* 2004). Mariod *et al.* (2009) investigated the oxidative stability of marula oil and compared it with sesame, peanut, sunflower, and cottonseed oils, which are conventional edible oils consumed in Sudan. They reported significantly ($P \leq 0.05$) higher stability of marula oil over conventional oils.

Phenolic compounds from *Sclerocarya birrea* oil seed cake extracted by overnight and ultrasound extraction resulted in a higher amount of total phenolic compounds. The addition of the extracts obtained from sunflower oil showed an inhibition of oxidation and a remarkable antioxidative activity, reducing oil deterioration (Mariod *et al.,* 2006). Crude oils obtained by oilseed processing have to be refined before consumption in order to remove

undesirable accompanying substances, such as free fatty acids, phosphoacylglycerols, sterols, pigments, glucosides, waxes, and hydrocarbons (Pokorný, 2001).

Antioxidant activity and stability of oils may be related to their fatty acid, tocopherols, and phenolic compounds. The antioxidant activity of 3,4-dihydroxyphenylethanoland andphenyl acids (caffeic acid, *p*-coumaric acid, ferulic acid, syringic acid, and vanillic acid)that are found in virgin olive oil has been studied, and their high antioxidant activity has been demonstrated (Baldioli *et al.*, 1996).

2.3. Pharmacology of *Sclerocarya birrea*

In view of its wide range of medicinal uses, *Sclerocarya birrea* has undergone extensive biological studies like antioxidant activity, antidiabetic properties, antagonistic effect, antiplasmodial and antimalarial activities, etc. and many studies have been performed on the basis of its chemical constituents and traditional uses.

2.3.1. Antioxidant activity

Research has pointed out that an effective method to reduce oxidative stress is antioxidant supplementation. When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (Jadhav *et al.*, 1996). The methanolic extracts from *Sclerocarya birrea* leaves, roots, barks, and kernel oil cake were examined for radical-scavenging capacities and antioxidant activities. The extracts showed high total phenolic compounds and they were markedly effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β-carotene in comparison with the control. Based on these findings, the seedcake extract was found to be the most effective, followed by the root, leaf, and bark extracts. Similarly, the antioxidant activity determined by the DPPH (2,2-diphenyl-β-picrylhydrazyl) method revealed that the seedcake extract had the highest antioxidant activity(Mariod *et al.*, 2008).

Sclerocarya birrea (A. Rich.) Hochst.juice was found to be a potent antioxidant, its effects were attributed to high contents of flavonoids and polyphenolic compounds (Borochov-Neori *et al.*, 2008). These phenolic compounds, besides having high antioxidant and free radical-

scavenging activities, appear to regulate signaling pathways involved in cellular survival, growth, and differentiation (Rice-Evans, 2001). Thus, diets with a high content of such phenolic-rich antioxidants emerge as a promising approach to help strengthen the physiological antioxidant defense system and to improve chronic diseases. The marula fruit juice, with its high antioxidative capacity, is a potential candidate for this approach (Mariod and Abdelwahab, 2012).

2.3.2. Antidiabetic activity

Sclerocarya birrea is most widely studied with regard to its antidiabetic effect and the plant has shown hypoglycemic activity(Braca *et al.,* 2003; Dimo *et al.,* 2007; Dieye *et al.,* 2008; Gondwe *et al.,* 2008). The stem-bark ethanol extract was found to be used as complementary remedy in diabetes (Musabayane *et al.,* 2006). *S. birrea* was evaluated for the treatment of type II diabetes(2) using in vitro models. Complications are frequently encountered in diabetes and these are associated with irreversible functional and structural changes in various organs, particularly the kidneys, eyes, nerves, heart, and blood vessels (Borochov-Neori *et al.,* 2008). The extract caused significant reduction in blood pressure in anesthetized and conscious normal and diabetic rats (Musabayane *et al.,* 2006).

2.3.3. Anti-inflammatory and analgesic properties

Sclerocarya birrea is used in folk medicine for the treatment of inflammatory disorders (Ojewole, 2004; Fotio *et al.,* 2009). Ojewole (2003) evaluated the anti-inflammatory effect of stem-bark aqueous and methanolic extracts of *S. birrea*. Both the aqueous and methanolic extracts reduced rat paw edema induced by subplantar injections of fresh egg albumin, due to the inhibition of histamine and prostaglandin pathways and to its antioxidant activity, as indicated by the glutathione and malondialdehyde levels in rats.

2.3.4. Antiparasitic and antimicrobial activities

Several studies have shown the usability of medicinal plants in the treatment of trypanosomiasis, which causes economical and epidemiological hazards (Grover and Yadav, 2004;Mikail, 2009). The methanolic extract of *S. birrea* leaves and stem bark showed complete mortality of *Trypanosoma brucei brucei* in vitro (Mikail, 2009). Although complete

mortality of the organism was observed, these studies did not provide a mechanism by which this extract exhibit its effect nor was the active pure compound reported (Mikail, 2009; Jensen et al., 2008). Ethanol and water extracts of marula, which is used by South African traditionally to treat dysentery, also showed antiamebic activity when tested using the microtiter plate and *Entamoeba histolytica* (Fennell *et al.,* 2004) *S. birrea* was tested for in vitro antiplasmodial and in vivo antimalarial activities against *Plasmodium falciparum* and *Plasmodium berghei*, respectively. *P. falciparum* was more sensitive to the plant extracts than *P. berghei*. *S. birrea* methanol extract is more active than aqueous one (Gathirwa *et al.,* 2008).

2.3.5. Gastrointestinal and antihypertensive ctivities

S. birrea (A. Rich.)Hochst.is one of plant species used widely in traditional medicine in Africa against many diseases and affections, such as hypertension, dysentery, stomachache orgastroenteritis (Belemtougri *et al.,* 2001). Studies have been carried out on the effect of this plant on smooth andskeletal muscles.(Belemtougri *et al.,* 2007). The lyophilized decoction of this plant demonstrated antidiarrheic activity in experimental models of diarrhea induced by magnesium sulfate and sodium picosulfate (Garba *et al.,* 2006). On the other hand, the antispasmodic effect of *S. birrea* extract was studied on isolated rat duodenum where the extract has exhibited an inhibitory effect on the dose-response curves induced by acetylcholine (Ach) on rat duodenum and reduced the maximal response of Ach in a concentration-dependent manner (Belemtougri *et al.,* 2007).

2.3.6. Ethnonutritional and ethnomedicinal uses

Dried seeds and nuts are widely consumed by local populations in Africa, especially those who inhabit rural areas (Galvez *et al.,* 1992). In some African countries, the stem bark, roots, and leaves of *S. birrea* are used for an array of human ailments, including malaria and fevers, diarrhea and dysentery, stomach ailments, headaches, sore eyes, toothache, backache and body pains, infertility, schistosomiasis, constipation, abdominal cramps and some other unspecified gastrointestinal problems, toothaches and swollen or infected gums, cough, hypertension, arthritis, proctitis, epilepsy, diabetes mellitus, sores, boils, carbuncles, abscesses and certain other bacterial infections, etc.(Ojewole, 2003; Glew *et al.,* 2006). In East Africa,

roots are an ingredient in an alcoholic medicine taken to treat internal ailments, whereas the bark is used for stomach disorders (Van Wyk *et al.*, 1997).

3. MATERIALSAND METHODS

3.1. Description of Study Area

The study was carried out in Meyu Muluke *Woreda* (district). The district is one of the 20 Woredas in East Hararghe province, Oromia Regional State, Ethiopia. It is located at a distance of 150km from Harar town to the South direction. It has 19 kebeles of which 18 are rural kebeles and one is urban kebele known as Huse which is the administration town center of the woreda (Fig. 1). Meyu Muluke District lies between 7°32' and 8°54' N latitude and 41°39' and 42°11' E longitude to the south of Harar city. It is bordered by Girawa to the North, Gola Oda woreda to the West, Kumbi woreda to the south west, MidhegaTola woreda to the East and Somali regional state to the south.

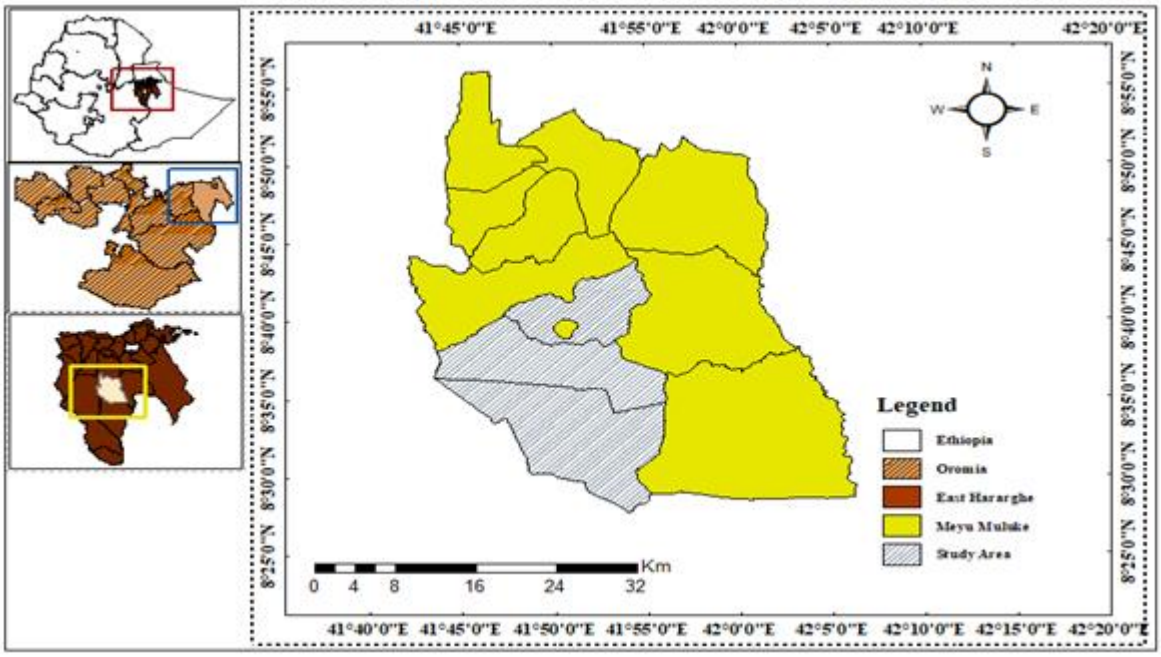

Figure 1: Map of the Study Area

The MeyuMuluke district's climatic condition is totally characterized by the lowland *Kolla (Gamoji)* climate. The study area experiences a bimodal rainfall pattern with two rainfall seasons, the Meher (Badheysa) rain (mid April-mid July) and the Kiremt (Ganna) rains (July-September) while the rest of the year remains dry. The annual rainfall of the Woreda ranges between 400mm and 600mm and its temperature is between 29 and 37^{0}C (Meyu Muluke woreda DPFSO, 2011).Meyu Muluke Woreda covers a total area of 4, 500 Km^2 (450,000

hectares). The total area of agricultural potential accounts for 20,154 hectares from the total area in which 9,254 ha(2.06%) of it is cultivated. Forest area covers 390,567 hectares which is the largest land use/cover of the woreda. Grazing land accounts for 11,925 hectares. The remaining 27,354 hectares of the woreda is covered by Settlement areas (MeyuMuluke Woreda PADO, 2010).

3.2. Study Design

Marula (*Sclerocarya birrea*) leaf and seed samples were collected from MeyuMuluke district. Qualitative analysis of major secondary metabolites including alkaloids, flavonoids, saponins, steroids, tannins and terpenoids of the*S. birrea* leaf and seed samples were carried out on dried and powdered plant specimens using standard methods. The antimicrobial experiment was arranged as a three factor experiment with two sources of extracts: leaf and seed of *S. birrea*; two solvents: water and methanol; four test pathogens including two bacteria: *E. coli* and *S. aureus*, and two fungi: *Aspergillus versicolor*, and*A. niger* in three replications. A complete randomized design (CRD) was used to determine the antimicrobial activities using the disc diffusion and broth dilution methods.

3.3. Collection of Plant Materials and Extract Preparation

Marula (*Sclerocarya birrea*) leaf and seed samples were collected from MeyuMuluke district, East Hararghe, Ethiopia. The authenticity of the plant material was confirmed in the Herbarium at Haramaya University. The fresh samples were washed with distilled water and residual moisture was evaporated at room temperature. The leaf and seed samples were cut into pieces then freeze dried. Thereafter, the samples were ground to a fine powder in a grinder for 2 min, the process was stopped for 15sec to avoid heating of the sample.

3.3.1. Preparation of the Extract

Fifty grams of the powdered plant material was soaked in 400 ml of 70% methanol in a conical flask sealed with aluminum foil and allowed to stand for 72 hrs with shaking. Then it was filtered by Whatman number 1 filter paper to obtain a solution of the crude extract. The

resulting alcoholic filtrate was concentrated using freeze dryer. After solvent evaporation, the remaining crude extract was kept in air tight bottle in a refrigerator until use at 4^{o}C.

3.3.2. Preparation of different concentrations of the crude extracts

The stock solution (200 mg/ml) was prepared by reconstituting 4g of each dried extracts in 20 of methanol. Different concentrations (100 mg/ml, 150mg/ml and 200mg/ml) of the extracts were prepared from their respective stocks. For preparing 100 mg/ml and 150mg/ml concentrations, 1g and 1.5g of the different stock solutions of the extracts were transferred, respectively, to separate volumetric flasks and the flasks were filled up to 10ml mark with methanol as per the method described by Mousavi *et al.* (2015).

3.4. Phytochemical Screening of the Plant Materials Extracts

Qualitative analysis of major secondary metabolites including alkaloids, flavonoids, saponins, steroids, tannins and terpenoids of the*S. birrea*leaf and seed samples were carried out on dried and powdered plant specimens using standard methods described by Evans (1996).

Detection of Alkaloids: 0.5g of the extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids using Mayer's test. Filtrates were treated with Mayer s reagent. Formation of yellow cream precipitate indicated the presence of alkaloids.

Detection of Saponins: about 0.5mg of the extract was shaken with 5ml of distilled water. Formation of froth (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids using Lead Acetate: 1ml of the extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicated the presence of flavonoids.

Detection of Tannins: a small quantity of extract was mixed with water and heated on a water bath. The mixture was then filtered and ferric chloride was added to the filtrate. Formation of a dark green color indicated the presence of tannins.

Tests for terpenoids using Salkowski's test: One ml of the extract was treated with 1ml of chloroform and filtered. The filtrate was mixed with few drops of concentrated sulphuric acid,

shaken and allowed to stand. If the lower layer turns red, a steroid is present. Presence of golden yellow layer at the bottom indicated the presence of triterpenoids.

Test for phlobatannins: 1 ml of each solid extract was placed into separate test tubes and mixed with 10 ml of distilled water. The mixture was boiled in a water bath for 10 min. Thereafter, 1ml aqueous hydrochloric acid was added to each mixture and shaken to develop red precipitate that indicates the presence of phlobatannins.

Test for steroids (Lieberman-Burchard's Test): 1 ml of chloroform and 10 drops of acetic acid were placed in a test tube. The concentrated extract (1 mg) was added to the test tube and mixed with the solvents. Then, 2 ml of concentrated sulphuric acid was added along the side of the test tube. The change of red color through blue to green serves as an indicator for the presence of steroids.

3.5. Antimicrobial Activity Test

The antimicrobial experiment was arranged in a 2x2x4 design which included twosourceof extracts: marula leaf and seed extracts at three concentration levels; two solvent system i.e. water and methanol; four test pathogens: two bacteria: *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive), two fungi: *Aspergillus versicolor* and *A. niger* in completely randomized factorial design in three replications. The test pathogens were obtained from Ethiopian Institute of Food and Public Health, Addis Ababa. The fungal and bacterial pathogens were subcultured and maintained on Potato Dextrose Agar (PDA) and Nutrient Agar, respectively. Then, the fungal and bacterial cultures were incubated for 72 h at 27 ºC and for 18-24 h at 37 ºC, respectively.

Media Preparation and Standardization of Inoculum: nutrient agar (NA), Potato Dextrose Agar (PDA), and Muller Hinton agar (MHA) were used for subculturing of bacterial test organism, fungal test organism, and determination of antimicrobial activities, respectively. These media were prepared and sterilized using an autoclave according to the manufacturers' instructions. The bacterial colonies and spores of the test fungi were harvested by washing the surface of the fungal colony using 5mL of sterile saline solution. This procedure was repeated until the turbidity of each bacterial and fungal spore suspension matched the turbidity of 0.5

McFarland Standards as described by the Clinical Laboratory Standards Institute (CLSI, 2015). The resulting suspension was used as inoculums for the test pathogen in the antimicrobial susceptibility test.

3.5.1. Measurement of diameter of inhibition zone

Disc diffusion Method: Discs of 6 mm diameter were prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then each disc was impregnated with 0.01 ml of the prepared test extract solution. The extract impregnated discs were placed onto MHA plates evenly inoculated with test pathogens (Hudzicki, 2009). Following this step, the impregnated discs were dispensed onto the surface of the MHA plates using sterile forceps (CLSI, 2015). Discs of commercial gentamycin (100mg/disc) and griseofulvin (100mg/disc) werealso used as positive controls for bacterial and fungal pathogens, respectively and distilled water soaked discs were used as negative controls. Then the MHA plates were sealed with parafilm and incubated at 37°C for 24 hrs and 27°C for 72 hrs for bacterial and fungal pathogens, respectively. The diameters of the zone of inhibition around each disc was measured to the nearest millimeter along two axes (i.e. 90° to each other) using a transparent ruler and the means of the two readings were recorded.

3.5.2. Determination of minimum inhibitory concentration

The marula extracts that showed significant antimicrobial activity in the antimicrobial activity tests were selected for determination of MIC using the broth dilution method. Accordingly, two mililiter of nutrient broth and potato dextrose broth for bacteria and fungi respectively was added into all test tubes and 0. 1 ml of the prepared concentration of each extract was mixed with the nutrient broth and potato dextrose. Thereafter, standardized inoculums of 0.1 ml of the respective test pathogens were dispensed into the test tubes containing the suspensions of the broth and the crude extract. Then, all test tubes were properly corked with aluminum foil and incubated at 37°C for 24 hrs for bacteria and 27°C for 72 hrs for fungi. After that, they were observed for absence or presence of visible growth. The lowest concentration at which no visible growth of organisms was regarded as the MIC (CLSI, 2012).

3.6. Antioxidant Activities

3.6.1. Antioxidant activity test by DPPH method

Antioxidant activity was tested as a release of the stable DPPH radical. The reaction mixture (5 ml) contained in 1 ml of 0.4 mM DPPH (15.8 mg DPPH in 100 ml of methanol) and 4ml of test extract solution dissolved in methanol. After incubation for 30 minutes at 37°C in the dark room, absorbance was measured at λ 515 nm using UV-Vis spectrophotometer. Lower absorbance indicates higher restriction of free radical scavenging (% inhibition) (Kochhar and Rossell, 1990).

3.6.2. Hydrogen peroxide scavenging ($_{H2O2}$) assay

The ability of fungal extracts to scavenge hydrogen peroxide was estimated by following the method of Ruch *et al* (1989) that a solution of hydrogen peroxide (40 mmol/L)was prepared in phosphate buffer (50 mmol/L, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm. Endophytic fungal extracts (1 mg/ml) in distilled water was added to hydrogen peroxide and absorbance at 230 nm by using a spectrophotometer and was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated as follows:

Scavenged $_{H2O2}$ (%) = [(Ai -At)/Ai]× 100

Where *Ai* is the absorbance of control and At is the absorbance of test samples.

3.7. Data Analysis

All data were entered into Microsoft excel. Mean comparison and Analysis of variance (ANOVA) werecarried out using SAS version 9.4 software package. Statistically significant differences were indicated by $p<0.05$.

4. RESULTS AND DISCUSSION

4.1. Phytochemical Screening of Marula *(Sclerocarya birrea*) Leaf and Seed Extracts

The phytochemical composition of aqueous and methanolicextract of *S. birrea* leaf and seed is presented in Table 1. The leaf aqueous extract has revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and steroids while alkaloids, saponins, flavonoids, terpenoids, tannins, and steroids were detected in methanolic leaf extract of *S. birrea*. As the seed aqueous extract demonstrated the presence of alkaloid, flavonoids, tannins, and steroids. The methanolic seed extract has demonstrated the presence of alkaloids, saponins,flavonoids, tannins and steroidsas the active phytoconstituents. The presence of all these major phytochemicals suggest that the strong biological activities and as source of antioxidants and antimicrobial potential of this plant. This finding was in accordance with Manzo et al (2017) who repored that the phytochemical screening of *S. birrea* also revealed the presence of flavonoid, saponin and tannin in all the plant extracts. The extract of *Sclerocarya birrea* (marula) stem bark indicated the presence of tannin, saponins and alkaloids with the absence of flavonoids, steroids and glycosides (Mai *et al.,* 2019).

Table 1. Phytochemical Screening of *S.birrea* Leaf and Seed Extracts

Plant parts	Solvent	Major chemical constituents						
		Alkaloid	Saponins	Flavonoids	Terpeniods	Tannins	Phlobatannins	Steroid
Leaf	Aqueous	+	-	+	+	++	-	+
	Methanol	+	+	+	+	++	-	+
Seed	Aqueous	++	-	+	-	+	-	++
	Methanol	++	+	+	-	+	-	++

(++): highly detected; (+): detected; (-): not detected.

Phenolic compounds, including flavonoids, anthocyanins and tannins, are the main group of antioxidant phytochemicals with interesting properties and have great value due to their biological and free radical scavenging activities (Elfalleh *et al.,* 2011). Polyphenol components found in all fruits and vegetables play a major role in many biological activities like colour, flavor, texture as well as antioxidant and antibacterial activities.

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs (Rhoades, 1979). Flavonoids enhance the effects of Vitamin C and also known to be biologically active against, tumors, and other microbes (Korkina *et al*., 1997). Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal sonedies and are under investigation for Antibacterial, and other Pharmaceutical functions (Yamunadevi *et al*., 2011). Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects.

Terpenoids have been implicated in antibacterial and antineoplastic functions hence the use of the plant to treat burns, skin diseases and insect stings (Yasoubi *et al.,* 2007). The presence of flavanoids is indicative of its potential use as an anti–allergic, anti inflammatory, anti oxidative, antimicrobial anti diarrhea and anticancer (Hajdu and Hohmann, 2012). Alkaloids are known with its pharmacological use for producing analgesics, stimulants, antihypertensive, anticancer, antibacterial, antiarryhthmia, antiasthma, antimalarial and recreational drugs (Jayaprakash, 2017).

4.2. Antioxidant Activities of *S. birrea* Leaf and Seed Extracts

The antioxidant activity of the *S. birrea* crude extract was measured based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide free radical scavenging activities (Table 2). The highest DPPH radical scavenging activity (39.25%), hydrogen peroxide scavenging activity (17.77%) were recorded for aqueous seed extract. By contrast, the least DPPH radical scavenging activity (29.32%) and hydrogen peroxide scavenging activity (5.40%) were recorded for methanolic leaf extract. It was observed that aqueous extract has better radical scavenging activity than crude methanolic extract of *S. birrea* leaf and seed. This finding was in accordance with previous works (Rice-Evans, 2001; Mariod and Abdelwahab, 2012).

Table 2.Antioxidant activityes of S. birrea leaf and seed crude extracts

Source	Solvent	DPPH (%)	HPSA (%)
Leaf	Aqueous	31.63c	11.23c
	Methanol	29.32d	5.40d
Seed	Aqueous	39.25a	17.77a
	Methanol	35.82b	14.24b

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column.

Antioxidant molecules can be classified in different ways depending on their environment and the functions they perform. An antioxidant is defined as a substance that can significantly delay or completely prevent the oxidation of substrate molecules, even at low concentrations (Gulcin *et al.,* 2010). They donate electrons to free radicals, rendering them harmless, and neutralize them by minimizing oxidative damage in biological processes. Antioxidants prevent free radical formation by interfering with the free radical-mediated oxidative process at any of its three main stages: initiation, propagation, and termination. The use of a free DPPH is the most common method (Kedare and Sing, 2011).

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPHH and as a consequence its absorbance decreases from that of the DPPH. Change of the radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. The more the decolorization the more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then it gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour (although it would be expected to get a residual pale yellow colour from the picryl group still present) (Handa *et al.,* 2006).

4.3. Antimicrobial Activities of Methanolic Extracts of *S. birrea* Leaf and Seed Crude Extracts

The antimicrobial activities of aqueous and methanolic extracts from *S. birrea* leaf and seed extracts based on disc diffusion method is shownin Table 3. The mean zones of inhibition of all extracts have revealed considerable antimicrobial activities. The antibacterial activity of aqueous extracts of*S. birrea*leaf and seed at highest concentration of the extracts has recorded mean zone of inhibition ranging from 13.67 to 15.73mm while the antibacterial activity of the methanolic extract with colony growth inhibitory effect at the highest dose showed a mean zone of inhibition ranging from14.50 to 18.53mm. In most cases, the susceptibility of the tested bacteria to the crude extracts have demonstrated medium to higher antibacterial activities. This finding was in agreement with that of Manzo *et al* (2017) who suggested that the extracts from the different parts of *S. birrea* showed varied antibacterial activity against the test bacteria. The bark extracts showed superior activity against *Escherichia coli* (zone of inhibition = 17 mm) and *Salmonella typhi* (zone of inhibition = 20 mm) at 200 mg/ml.

Table 3. Antibacterial activity of the crude extracts based on diameter of inhibition zone

Test pathogens	Source	Solvents	Concentration of the extracts (mg/ml)			Gentamycin
			100	150	200	(100mg/ml)
E. coli	Leaf	Aqueous	8.50eD	11.50cC	14.17cB	19.83Aa
		Methanol	10.50cD	14.50bC	16.20bB	19.67aA
	Seed	Aqueous	7.50fD	11.83cC	13.67cB	19.53aA
		Methanol	9.50dD	11.50cC	14.50cB	19.17aA
S. aureus	Leaf	Aqueous	11.53bD	13.73bC	15.73bB	19.50aA
		Methanol	12.50aC	15.83aB	18.53aA	19.67aA
	Seed	Aqueous	10.83bcC	11.60cC	14.43cB	19.00aA
		Methanol	11.50bD	13.97bC	16.00bB	19.33aA

Means followed by same letter within a column androw were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row.

In most of the extracts Gentamycin (used as positive control) showed a significant superiority ($p<0.05$) in the zone of inhibition as compared to the test extracts (Table 3). The superiority of antibiotics might be due to the method of extraction and the type of solvent used for the extraction. For most of the test extracts, the highest concentration (200mg/ml) exhibited a significantly higher ($p<0.05$) zone of inhibition as compared to the respective lower concentrations (100mg/ml & 150mg/ml). The highest concentration of the leaf methanolic extract (200mg/ml) presented the strongest antibacterial activity with maximum zone of inhibition (18.53mm) against *S. aureus* indicating that *S. aureus* is more susceptible to the crude extract than *E. coli*.On the other hand, The seed aqueous extract has presented the weakest antibacterial activity with minimum inhibition zone of 13.67mm against *E. coli*,indicating that *E. coli* was the most resistant to the antimicrobial seed extract.

The antifungal activity of aqueous extracts of *S. birrea* leaf and seed at highest concentration of the extracts showeda mean zone of inhibition ranging from 9.83 to 15.53 mm while the antifungal activity of the methanolic extract showed a mean zone of inhibition ranging from16.50 to 19.93mm (Table 4). The highest concentration of the extract revealed maximum antifungal activity with the highest zone of inhibition (19.93 mm) was recorded for methanolic leaf extract against *A. versicolor*. This indicatedthat *A. versicolor* was moresusceptible than the other tested fungal pathogens whereas the weakest antifungal activity with minimum zone of inhibition (9.83 mm) (at the highest concentration of the extract) was recorded for aqueous seed extract against *A. niger*.Asimilar study was conducted by Mai et *al*. (2019) who reported that the extracts with trona have higher zone of inhibition at 200 mg/ml, *Escherichia coli*,(zone of inhibition = 16), *Staphylococcus aureus* (zone of inhibition = 14) and *Klebsiella pneumoniae* (zone of inhibition = 9).

Table 4. Antifungal activity of crude extracts based on diameter of inhibition zone

Test pathogens	Source	Solvent	Concentration of the extracts (mg/ml)			Griseofulvin (100mg/ml)
			100	150	200	
A. niger	Leaf	Aqueous	6.90eC	8.17eC	11.50eB	18.97aA
		Methanol	11.30aC	15.50aB	18.57aA	20.10aA
	Seed	Aqueous	6.67eD	8.10Ec	9.83fB	19.90aA
		Methanol	9.53dD	14.00bC	16.50bcB	19.17aA
A. versicolor	Leaf	Aqueous	9.50dD	11.47dC	15.53cdB	19.40aA
		Methanol	10.93abD	13.70bC	17.17bB	19.53aA
	Seed	Aqueous	9.97cdD	12.83cC	14.53dB	19.87aA
		Methanol	10.50bcC	12.50cB	19.93aA	19.80aA

Means followed by same letter within a column androw were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row.

4.4.MinimumInhibitory Concentration (MIC),Minimum Bactericidal Concentration (MBC), &Minimum Fungicidal Concentration (MFC) of the Crude Extracts of *S. birrea*

The crude extracts were further tested for MIC, MBC and MFC. The results of the aqueous and methanolic extracts of *S. birrea* leaf and seed against test pathogenic are shown in Table 5. It was observed that the methanolic extract from leaf and seed presented strongest antibacterial activity with MIC of 3.13 mg/ml(the least value) and the corresponding MBC of 3.13mg/ml against *S.aureus* while the weakest antibacterial activity with MIC of 150 mg/ml (the largest value) and the corresponding MBC of 300mg/ml was observed for *S. birrea* aqueous seed extract against *E. coli.*Thus,on the basis of this resultit can be concluded that methanolic leaf extract of *S. birrea* possessed the strongest antibacterial potential. Furthermore, *S. aureus* was more susceptible than *E.coli* to the antibacterial extract while *A versicolor* was more susceptible than A.niger to the antifungal extract of *S. birrea*leaf and seed crude extracts.

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) of aqueousand methanolic extracts

Test pathogens	Source	Solvent	MIC (mg/ml)	MBC/MFC (mg/ml)
E. coli	Leaf	Aqueous	100	200
		Methanol	50	100
	Seed	Aqueous	150	300
		Methanol	100	200
S. aureus	Leaf	Aqueous	25	50
		Methanol	3.13	3.13
	Seed	Aqueous	75	200
		Methanol	50	100
A. niger	Leaf	Aqueous	12.5	25
		Methanol	25	75
	Seed	Aqueous	150	300
		Methanol	100	200
A. versicolor	Leaf	Aqueous	50	100
		Methanol	6.25	6.25
	Seed	Aqueous	50	100
		Methanol	25	50

The strongest antifungal activity with MIC (6.25mg/ml) and the corresponding MFC (6.25mg/ml) was recorded for methanolic extract of*S. birrea* leaf against *A. versicolor* whereas the weakest antifungal activity with largest MIC (150mg/ml) and the corresponding MFC (300mg/ml) was observed for aqueous seed extract against *A. niger* indicating that *A. niger* was the most resistant, while *A. versiclor* was the most susceptible to the crude extract of *S. birrea* leaf and seed extracts.The present study is in agreement with those of antibacterial and phytochemical studies on *S. birrea* against various enteropathogens that are implicated in the development of diarrhea and/or other gastrointestinal disorders. Simialr work was reported by Atto et al (2016) who investigated phytochemical Screening of *Sclerocarya birrea*(Anacardiaceae) and Khaya senegalensis (Meliaceae), antidiabetic plants.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

Marula(*Sclerocarya birrea* (A. Rich.)Hochst.)is often referred to as the “tree of life “since its leaves, stem bark, root, and fruits are used in food and traditional medicine.The seed is rich in protein and oil, magnesium, phosphorus and potassium, which make it important to an African diet. The seeds are eaten, dried or ground and added to soups, stews and vegetables, to which they are reputed to give a delicious flavour. Fresh seeds are also added to newly–boiled meat, which is then eaten immediately. The *S. birrea* plant is distributed in low and midaltitude regions of East Hararghe, Ethiopia. Therefore, the present study is undertaken to investigate antioxidant and antimicrobial activities of aqueous and methanolic extracts of *S. birrea* seed and leaf extracts.

The marula (*Sclerocarya birrea*) leaf, and seed samples were collected from MeyuMuluke district. Qualitative analysis of major secondary metabolites including alkaloids, flavonoids, saponins, steroids, tannins and terpenoids of the*S. birrea* leaf and seed samples were carried out on dried and powdered plant specimens using standard methods. The antimicrobial experiment was arranged in a three factor experiment with two source extracts: leaf and seed of *S. birrea*; two solvents: water and methanol; four test pathogens including two bacteria: *E. coli* and *S. aureus*, and two fungi: *Aspergillus versicolor*, and*A. niger* in three replications.

The leaf aqueous extract has revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and steroids while alkaloids, saponins, flavonoids, terpenoids, tannins, and steroids were detected in methanolic leaf extract of *S. birrea*. As the seed aqueous extract demonstrated the presence of alkaloid, flavonoids, tannins, and steroids. The methanolic seed extract has demonstrated the presence of alkaloids, saponins, flavonoids, tannins and steroids as the active phytoconstituents.

The antioxidant activity of the *S. birrea* crude extract was measured based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) indicated the highest DPPH radical scavenging activity (39.25%), hydrogen peroxide scavenging activity (17.77%) were recorded for aqueous seed extract.

The highest concentration of the leaf methanolic extract (200mg/ml) presented the strongest antibacterial activity with maximum zone of inhibition (18.53mm) against *S. aureus* indicating that *S. aureus* is more susceptible to the crude extract than *E. coli*. On the other hand, The seed aqueous extract has presented the weakest antibacterial activity with minimum inhibition zone of 13.67mm against *E. coli*, indicating that *E. coli* was the most resistant to the antimicrobial seed extract.

The highest concentration of the extract revealed maximum antifungal activity with the highest zone of inhibition (19.93 mm) recorded for methanolic seed extract against *A. versicolor*. This indicate that *A. versicolor* was more susceptible among tested fungal pathogens whereas the weakest antifungal activity with minimum zone of inhibition (9.83 mm) (at the highest concentration of the extract) was recorded for aqueous seed extract against *A. niger*.

The methanolic extract from leaf and seed presented strongest antibacterial activity with MIC of 3.13 mg/ml(the least value) and the corresponding MBC of 3.13mg/ml against *S.aureus* while the weakest antibacterial activity with MIC of 150 mg/ml (the largest value) and the corresponding MBC of 300mg/mlwas observed for *S. birrea* aqueousseed extract against *E. coli*. Thus,on the basis of this resultit can be concluded from this result that methanolic leaf extract of*S. birrea* possessed the strongest antibacterial potential. Furthermore, *S. aureus* was more susceptible than*the E.coli* to the antibacterial activity while *A. versicolor* was more susceptible than A.niger to the antifungal activity of the *S. birrea* crude extracts.

The strongest antifungal activity with MIC of 6.25mg/ml and the corresponding MFC of 6.25mg/ml was recorded for methanolic extract of *S. birrea* leaf methanolic extract against *A. versicolor* whereas the weakest antifungal activity with largest MIC of 150mg/mland the corresponding MFC of 300mg/ml was observed for aqueous seed extract against *A. niger* indicating that *A. niger* was the most resistant, while *A. versiclor* was the most susceptible to the crude extract of *S. birrea* leaf and seed extracts.

5.2. Conclusion

It can be concluded from the result of the present study that the presence of major phytochemicals suggestthe strong biological activities and as source of antioxidants and antimicrobial potential of this plant.It was observed that aqueous extract has better radical scavenging activity than crude methanolic extract of *S. birrea* leaf and seed.The susceptibility of the tested bacteria to the crude extracts have demonstrated medium to higher antibacterial activities. The methanolic leaf extract of *S. birrea* possessed strongest antimicrobial potential. *S. aureus* was the more susceptible to the antibacterial activity while *A versicolor* was more susceptible to the antifungal activity of the *S. birrea* crude extracts.

5.3. Recommendations

- Phytochemical analysis of *S. birrea* leaf and seed extracts revealed the presence of tannins, phlobatannins (a condensed form of tannin), saponins, flavonoids, terpenoids, steroids and alkaloids using aqueous and methanol solvents. Thus, using different solvent extractions for bioactive compounds are recommended as bioactive compounds are solvent dependent;
- More specific methods can be used for detection of MIC e.g. Micro-dilution method and agar well diffusion methods;
- The*S. birrea* leaf and seed extracts showed antimicrobial activities against common pathogenic microbes. Further *in vivo* studies including cell toxicity assay are recommended;
- The present study focused only qualitative screening of phytochemicals. However, structural elucidation of the active principles of phytochemicals need to be conducted.

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7. APPENDIX

Appendix Table 1. Antioxidant activity of the aqueous and methanolic leaf and seed crude extracts

Source	Solvent	Rep	DPPH (%)	HPSA (%)
Leaf	aqueous	1	31.09489	11.47208
	aqueous	2	32.16292	10.9879
	methanol	1	28.88483	5.734767
	methanol	2	29.75779	5.072464
Seed	aqueous	1	39.3534	17.05686
	aqueous	2	39.15289	18.49174
	methanol	1	35.12881	14.70588
	methanol	2	36.51877	13.76564

Appendix Table 2. Antibacterial activity of the S. birrea leaf and seed extracts

Test pathogens	Source	Solvent	rep	Concentration of the extract (mg/ml)			Gentamycin (100mg/ml)
				100	150	200	
E. coli	Leaf	Aqueous	1	8	11	15	20
		Aqueous	2	8.5	12	14	19.5
		Aqueous	3	9	11.5	13.5	20
		methanol	1	10.5	14.5	16.5	19
		methanol	2	10	13.8	16.1	20
		methanol	3	11	15.2	16	20
	Seed	Aqueous	1	8	12	13	19
		Aqueous	2	7	11.5	13.5	19.6
		Aqueous	3	7.5	12	14.5	20
		methanol	1	9	11	14	19
		methanol	2	9.5	11.5	14.5	19.5
		methanol	3	10	12	15	19
S. aureus	Leaf	Aqueous	1	11	14	16	20
		Aqueous	2	12	14.2	15	19.5
		Aqueous	3	11.6	13	16.2	19
		methanol	1	12	15	18	20
		methanol	2	12.5	16	19	19
		methanol	3	13	16.5	18.6	20
	Seed	Aqueous	1	10	12	15	18.5
		Aqueous	2	11	11	14.3	19
		Aqueous	3	11.5	11.8	14	19.5
		methanol	1	11	14	16	19.5
		methanol	2	11.5	13.5	16.5	19.5
		methanol	3	12	14.4	15.5	19

Appendix Table 3. Antifungal activity of the S. birrea leaf and seed aqueous and methanolic extracts

Test pathogens	Source	Solvent	Rep	Concentration of the crude extract (mg/ml)			Griseofulvin (100mg/ml)
				100	150	200	
A. niger	Leaf	Aqueous	1	7	8.5	11	19
		Aqueous	2	6.8	8	11.5	18.9
		Aqueous	3	6.9	8	12	19
		methanol	1	11	15	19	19.8
		methanol	2	11.5	16	19.7	20
		methanol	3	11.4	15.5	20	20.5
	Seed	Aqueous	1	7	7.8	9	20
		Aqueous	2	6	8	10.5	19.7
		Aqueous	3	7	8.5	10	20
		methanol	1	10	14	16	19
		methanol	2	9.6	14.5	17	19.5
		methanol	3	9	13.5	16.5	19
A.versicolor	Leaf	Aqueous	1	9	11	15	19.5
		Aqueous	2	9.5	12	15.6	20
		Aqueous	3	10	11.4	16	18.7
		methanol	1	11	13.5	17	20
		methanol	2	10.6	14	16.7	19
		methanol	3	11.2	13.6	17.8	19.6
	Seed	Aqueous	1	10	12	14	20
		Aqueous	2	9.8	13.5	15	19.6
		Aqueous	3	10.1	13	14.6	20
		methanol	1	11	12	18	19.8
		methanol	2	10.5	12.5	19.8	20
		methanol	3	10	13	19	19.6