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A comparative study on antibacterial effects of *Hagenia abyssinica* oil extracted from different parts of the plant using different solvents against two selected and standardized human pathogens

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Hagenia abyssinica **is an important medicinal plant that the traditional society relied on for generations as a traditional medicine for various types of worm infections. The tree is a multipurpose dioecious tree and can grow up to 20 m in length. This research work is designed to investigate the antibacterial activity of *H. abyssinica* oils extracted using three different kinds of solvent and from three different parts of the plant on two standard bacterial isolates obtained from Pasteur Institute located at Addis Ababa, Ethiopia. The solvents used to extract the oils are ethyl acetate, n-hexane and methanol. Accordingly, the antibacterial activity of all the oils were tested and the oil extracted using methanol from all parts of the plants was characterized by having a higher mean of zone of inhibition (which is 1.710 in centimeter diameter) as compared to the two other oils (n-hexane, which is 0910 and that of ethyl acetate, which is 0.842). The data obtained from this research work showed that the oils extracted from the different parts of the plant using the three different solvents exhibited a higher mean zone of inhibition on *Staphylococcus aureus* (which is 1.279) than that of *Escherichia coli* (which is 1.029). In addition, oil extracted from root of the plant has a higher mean of zone of inhibition (which is 1.415) as compared to the leaf (which is 1.14) and bark (which is 0.908). Generally, oils extracted from root, leaf and bark of *H. abyssinica* have an antibacterial property even if they exhibit difference.**

Key words: *Hagenia abyssinica,* oil, solvents, antibacterial activity, zone of inhibition, *S. aureus, E. coli.*

INTRODUCTION

Medicinal plants have been used since antiquity to treat various health problems, and about 80% of the Ethiopian people rely on traditional medicine to meet their healthcare needs (Endashaw, 2007). The widespread use of traditional medicine could be attributed to cultural acceptability, perceived efficacy against certain types of diseases, physical accessibility and affordability as compared to modern medicine (Girma, 1998; Debela et

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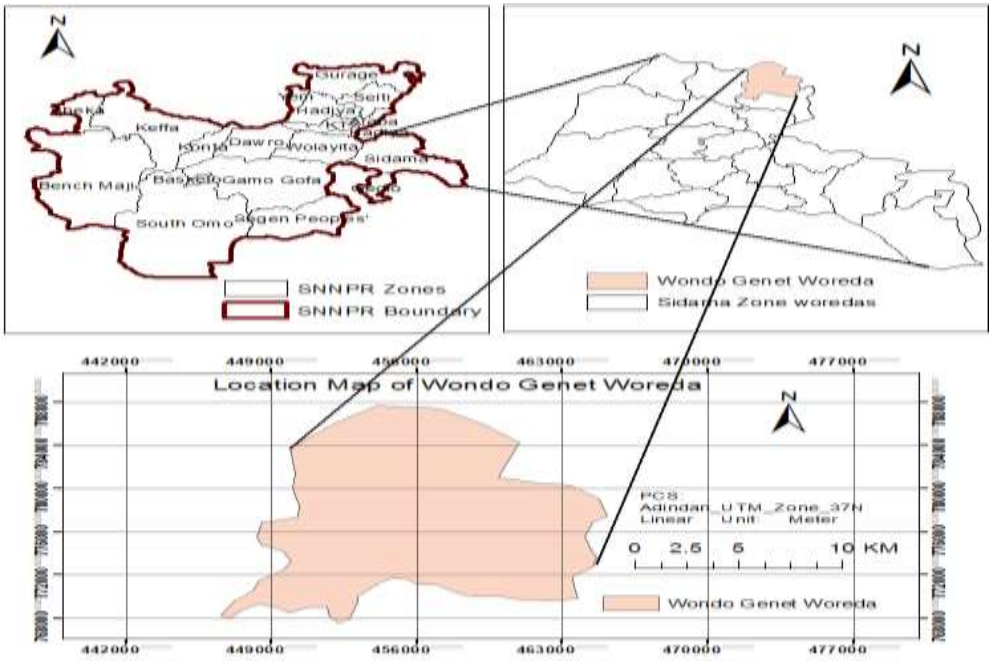

Figure 1. Map showing the location of the study area.

al., 2006; Endashaw, 2007).

Hagenia abyssinica is one of the key medicinal plants which people relied upon for generations to get-rid of various ailments. The species was once abundant in the semi-humid mountain woodlands of Ethiopia with altitudinal range between 2450 and 3250 m above sea level (Hedberg, 1989), but now the species is endangered and sparsely distributed due to over exploitation (Legesse, 1995). *H.abyssinica* is a multi-purpose tree that has every part of it used for different purposes such as medicine, timber, firewood, poles, mulch, green manure, and as an ornamental plant. The species has been widely used for its potent anti-tapeworm activity (Tileye, 2006; Biruktayet et al., 2010). Despite its endangered state and high call for conservation, detailed information on the agronomic and chemical traits of the species is lacking. Proper utilization and conservation efforts have not been established to take all the necessary actions to rescue this species.

Previous ethno-botanical studies on medicinal plants focused on the free listing of traditional medicinal plant species. To this end, medicinal plant, shrubs and trees, which are currently getting acceptance in herbal medicines of the country and also possessing higher genetic endemism such as *H. abyssinica* need to be considered for pharmacological analysis. The purpose of the present study is, therefore, to investigate the antibacterial effect of extracts of *H. abyssinica* on human pathogens.

The general objective of the study was to conduct a comparative study on the antibacterial effects of *H. abyssinica* oil extracted from different parts of the plant using different solvents against two selected and standardized human pathogens. The oils extracted from the different parts of the plant using the three different solvents were assessed for their antibacterial activity of against two selected and standardized human pathogens namely *Staphylococcus aureus* ATCC 25923 and *Escherchia coli* ATCC 25922. In addition, the research work has an aim of identifying which parts of *H. abyssinica is* most effective against the activity *of S. aureus* ATCC 25923 and *E. coli* ATCC 25922 and also intended to identify the effective solvent used for oil extraction from different parts of *H. abyssicnica* on the antibacterial action of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

MATERIALS AND METHODS

Study area

Wondo Genet Area is located in Wondo Genet Woreda, Southern Nations Nationalities and Peoples Regional State. This area is located 265 km away from Addis Ababa, the capital of Ethiopia. It is part of the Sidama Zone, located in the Great Rift Valley and is bordered on the south by Malga, on the west by Awasa Zuria, and on the north and east by the Oromia Region. The Woreda is located between 5°30′ N to 7° 20′ N latitude and 37° 05’ E to 39° 50’ E longitude and the elevation ranges from 1680 to 3960 m above sea level (Bizuwerk, 2004). The *H. abyssinica* plant samples were collected from the field and brought to the laboratory for extraction (Figure 1).

The data collection for this experimental research work consists of two major experimental steps. The first was the extraction of the crude oil from the different parts of the plant using different solvents namely methanol, ethyl acetate and n-hexane. The second

experimental step was testing the oils extracted from different parts of the plant using different solvents in response to the two selected reference species of bacteria namely *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

The experimental work manipulates three factors namely *H. abyssinica* parts (root, leaf and bark), different alcohol solvent (methanol, ethyl acetate and n-hexane) and two different species of standardized pathogenic bacteria species (*S. aureus* ATCC 25923 and *E. coli* ATCC 25922). The first two factors had three treatments and the third contains two species of standardized bacteria. The experiment has a total of eighty (18) treatment combinations each replicated four times to test the extent of growth inhibition on the two bacterial species namely *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

Hagenia abyssinica sample collection and oil extraction method

Healthy looking root, leaf and bark sample of *H. abyssinica was* collected from Wondo Genet College of Forestry and Natural Resources and transported to the laboratory ascetically. The bark and leaf parts were taken from medium position of the plant height whereas the root was taken from the lateral roots possibly because the traditional medicinal plant hunters used the matured parts of the plant for its medicinal purpose. The solvents used for extracting the oil were n-hexane, ethyle acetate and methanol (Hesham et al., 2016; Akinyemi et al., 2006).

The plant materials were collected; the fresh weight was recorded and dried using an oven dry method at the temperature of 40°C for three days. Thereafter, the fresh and dry weights of the plant part were measured using a digital balance. The dried sample was then crashed into smaller pieces using a clean laboratory cutting mill machine. Afterwards, 30 g of the dried sample was taken from the crushed and dried (leaf, root and bark) parts of the plant, was placed in a plastic bottle and these were diluted with 180 mL of ethyl acetate, n-Hexane and methanol. The bottles containing the solvents and dried sample were placed on an electrical mechanical shaker at 200 rpm. The solution was stayed on mechanical shaker for three successive days (Hesham et al., 2016; Akinyemi et al., 2006).

After three consecutive days of shaking, the solution was separated from the shaker and the residue was filtered with Whitman Filter Paper No 1. Then the liquid containing both the solvents and extract was placed in an oven drier adjusted at 30°C. In order to separate the solvent from the crude plant extract the solution was placed inside an oven drier for four consecutive days. After evaporation of the solvents from the crude oil extracts, the effectiveness of the crude oil extracted against bacteria types was tested according to the procedure of Hesham et al. (2016) and Akinyemi et al. (2006).

Test microorganisms and Inoculum preparation

Microorganisms used in the experiment are standard reference species with the American Type Culture Collection (ATCC) (*S. aureas* ATCC 25923 and *E. coli* ATCC 25922) obtained from Pasture Institute Laboratory located at Addis Ababa, Ethiopia. A Muller Hinton, McConkey and Nutrient agar were prepared and autoclaved at 121°C, 15 psi for 20 min. Thereafter, the autoclaved medias were placed inside a clean laminar flow cabinet, allowed to cool to 45°C, poured aseptically into sterilized petriplates and the plates were stayed overnight inside a clean laminar flow cabinet. The reference bacterial species which are stored in the Muller Hinton broth were aseptically inoculated using a spread plate method on the media by using a sterilized bent glass rod (Coyle, 2005; Akinyemi et al., 2006).

Antibiotic assay

The antibiotics diffusion method (Singh et al., 2006) was used to evaluate the antibacterial activity of the crude oils extracted using the three solvents. To activate growth, the standard reference species were cultured overnight at 37°C on Muller Hinton Broth and Nutrient Broth and the study organisms were inoculated on Nutrient agar and McConkey agar media. Sterilized discs of equal area were prepared using a clean cork borer and the discs were inoculated inside the extracted oils for more than three days until the discs absorbed the oil sufficiently in the different oils extracted from root, leaf and bark of the plant using different solvents in separate inside beakers (Akinyemi et al., 2006).

Four discs per plate from oil of each plant parts extracted using different solvents were inoculated on the media containing the standard reference species; the plates were then sealed with a parafilm and incubated at 37°C for 72 h. After incubation for three days, the plates were observed for the presence of clear inhibition zone around the discs. For each treatment, a replication was made and the zone of inhibition was measured in centimeters (Akinyemi et al., 2006).

Data analysis

After the necessary data collection was made, the data collected were organized and analyzed using SPSS version 16 and the findings were presented using tables and graphs. The means of zone of inhibition for the different oils extracted from the different plant parts using different solvents were compared using LSD test. Significant differences of the three factors were tested at α of 0.05. Finally, the result was presented in the form of tables, percentages and graphs.

RESULTS AND DISCUSSION

Effect of plant crude oil extract on bacterial growth

H. abyssinica is a plant commonly found in high altitude areas in East Africa. It is an important medicinal plant with the flower reported as antihypertensive, taenicidal (Desta, 1995), antihelminthic, trypanocidal (Nibert and Wink, 2010), and in treatment of eye disease (Abebe and Ayehu, 1993). *H. abyssinica* is a plant commonly found in high altitude areas in East Africa. It is an important medicinal plant with the flower reported as antihypertensive, taenicidal, (Desta, 1995), antihelminthic, trypanocidal (Nibert and Wink, 2010), and in treatment of eye disease (Abebe and Ayehu, 1993).

H. abyssinica is a medicinal plant commonly found in the highlands of East Africa and characterized by its medicinal property for various type of bacterial and worm infection by the local community for centuries (Lilian, 2013; Biruktayet et al., 2010). It has been serving the traditional community as an antihelmintic in ruminants and also against tapeworms in humans (Biruktayet et al., 2010); even the local name of the tree ‘koso’ refers to the tapeworm infecting human being. Wolde et al. (2016) has reported that the chemicals found inside the crude oil extract of the plant have an antibacterial property and the antibacterial compounds found inside the oils either kill or inhibit the growth of the inoculants. Accordingly, the crude oils extracted using the three different solvent and

Table 1. Mean zone of inhibition (± Standard Error) of the oils from different parts of the plant on *S. aureus* and *E. coli*.

Parts of the plant	Mean ± Std. Error
Root	1.415[a] ± 0.069
Leaf	1.140[b] ± 0.069
Bark	0.908[c] ± 0.069

Table 2. Mean zone of inhibition (± Standard Error) of the oils extracted using different solvent from different parts of *H. abyssinica* on the test organisms.

Solvent type	Mean ± Std. Error
Ethyl acetate	0.842 b ± 0.069
Hexane	0.910 b ± 0.069
Methanol	1.710 a ± 0.069

Table 3. Growth inhibition response of bacteria types for the extract of solvent and parts of *H. abyssinica*.

Bacteria type	Mean ± Std. Error
Staphylococcus aureus	1.279 a ± .057
Escherichia coli	1.029 b ± .057

from the different organ of the plant shows that they have an antibacterial activity even if their strength differ between solvents and parts of the plant. This is highly supported by the finding of Wolde et al. (2016) who stated that the presence of saponins, phenols and alkaloids could confer antibiotic property of the plant (Wolde et al., 2016).

The current study indicated that there is a considerable difference in the antibacterial activity of the oils extracted from different parts of the plant and extracted using different solvents (ethyl acetate, n-hexane and methanol). The oils extracted from root and leaf of *H. abyssinica* was more active in their antibacterial activity than the oil extracted from bark (Table 1). In addition to this, the oils extracted using methanol has a significant antibacterial activity than the oils extracted using ethyl acetate and n-Hexane (Table 1). This might be due to the high crude yield extract obtained using methanol as compared to ethyl acetate and n-hexane (Wolde et al., 2016).

Accordingly, besides interaction of parts of *H. abyssinica* with the selected bacterial species, the other three factors (solvent types, bacteria species types, parts of *H. abyssinica*) and the interactions (Plant Parts × Solvent types, Solvent × Bacteria types, and Plant Parts × solvent types × Bacteria types) were significantly different at $\alpha < 0.05$ (Tables 1, 2 and 3).

As shown in Table 1, the different parts of the study plant have different mean zone of inhibition (measured in centimeters) of bacterial growth. The root part of the study plant was characterized by having high zone of inhibition as compared to other parts of the plant and it is statistically significant at $\alpha = 0.05$ which is followed by leaf and bark.

The mean zone of inhibition while comparing the solvents showed that there is a difference with regard to inhibition of growth of the two selected species of bacteria. The oil extracted using methanol as a solvent resulted in higher mean zone inhibition (1.710) followed by n-hexane (0.910) and ethyl acetate (0.842) respectively on both species of bacterial isolates (Table 2).

The test on the two bacterial species showed that the two different bacteria had different mean of resistance to the extracted oil from the different parts of the plant. *S. aureus* result indicating higher mean of inhibition shows that it is relatively sensitive to the oils or have high inhibition zone as compared to *E. coli* (Table 3). This finding is in accordance with that of Wolde et al. (2016) which revealed that the largest zones of inhibition were recorded with methanol crude extract from *H. abyssinica* against *S. aureus.*

Interaction effect of solvents, parts of *H. abyssinica,* and bacteria types on the growth inhibition

As indicated in Figure 2, oil extracted from the root part of *H. abyssinica* using methanol as a solvent inhibit the growth of the two bacterial species better than that of leaf and bark extracts by the three solvent types (Ngeny et al., 2013). Oil extracted from bark part using hexane solvent resulted in lower mean zone of inhibition. This finding was also supported by Wolde et al. (2016) who stated that the antibacterial activity of methanol crude extract was better than ethanol extracts, and hexane.

When the means for the interaction effects for parts of the plant was carefully observed, the highest zone of inhibition was found on *S. aureus* than *E. coli* (Figure 3), indicating that *S. aureus* is highly sensitive to the crude oil extracts of *H. abyssinica* (Wolde et al., 2016, Ngeny et al., 2013). The root of *H. abyssinica* was recorded as having the highest zone of inhibition which was followed by the leaf and bark.

The study shows that the highest zone of inhibition from the interaction effect of the solvents and bacterial species was recorded for those oils extracted using methanol (1.725 for *S. aureus* and 1.696 for *E. coli*) followed by those oils extracted using hexane and ethyl acetate (Figure 4). When the relative mean of zone of inhibition in all the three solvents were compared, the highest mean of zone of inhibition was recorded on *S. aureus*. Similar finding by Wolde et al. (2016) and Ngeny et al. (2013) reported that *S. aureus* is sensitive to *H. abyssinica* crude oil extract.

The highest mean zone of inhibition by the oil extracted from root was recorded for the one which was extracted using methanol as a solvent (Figure 5). Similarly, the highest mean zone of inhibition by the oil extracted from

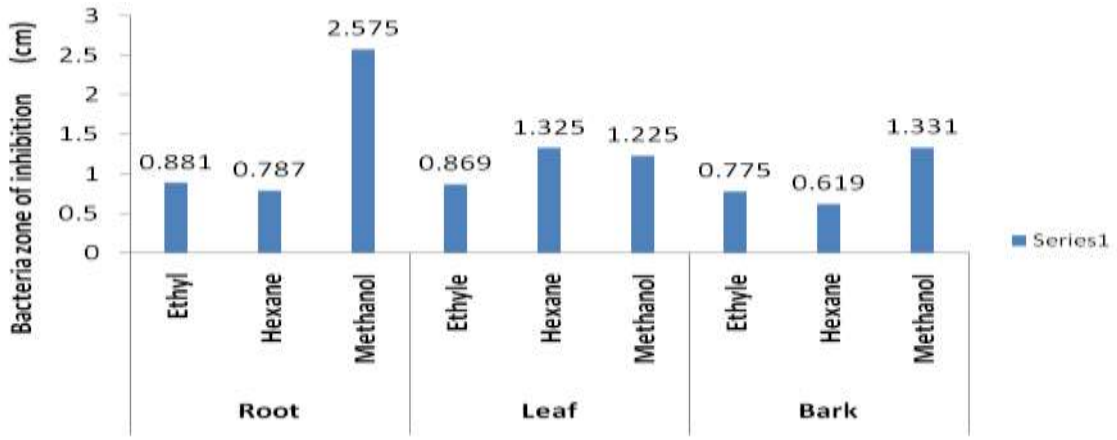

Figure 2. Interaction effects of parts of *H. abyssinica* and solvent types.

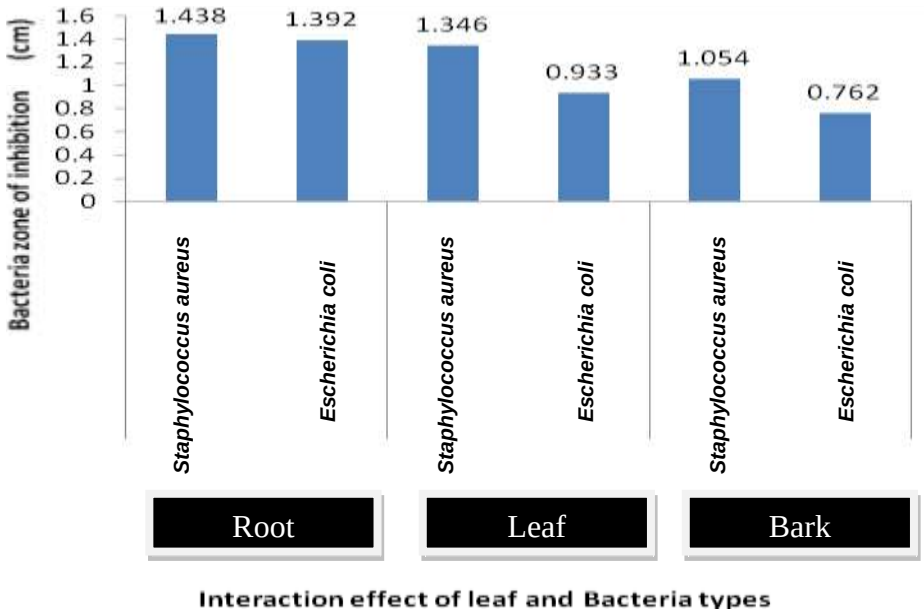

Figure 3. Interaction effects of parts of *H. abyssinica* on *S. aureus* and *E. coli*.

bark was recorded for the one which was extracted using methanol as a solvent. This might be the relatively higher crude oil extract gained by using methanol as a solvent, with similar finding also reported by Wolde et al. (2016) and Ngeny et al. (2013). However, the highest mean zone of inhibition by the oil extracted from leaf was recorded for the extract using hexane. In addition, the highest mean zone of inhibition was recorded on *S. aureus* (2.438) and *E. coli* (2.713) by the oil extracted using methanol and the root part of the plant followed by the oil extracted using methanol from bark of the plant (1.537 and 1.125, respectively) (Figure 5).

Conclusions

It was observed from this research work that oils extracted (using different solvents) from different parts (leaf, stem and bark) of *H. abyssinica* oils exhibited antibacterial property on *S. aureus* and *E.coli* with great difference in the diameter for zone of inhibition measured in centimeter. The oils extracted from the different parts of the plant has an antibacterial activity on the two selected study microorganisms even if there is difference in their strength between parts and between solvents.

While comparing the effectiveness of the oils extracted

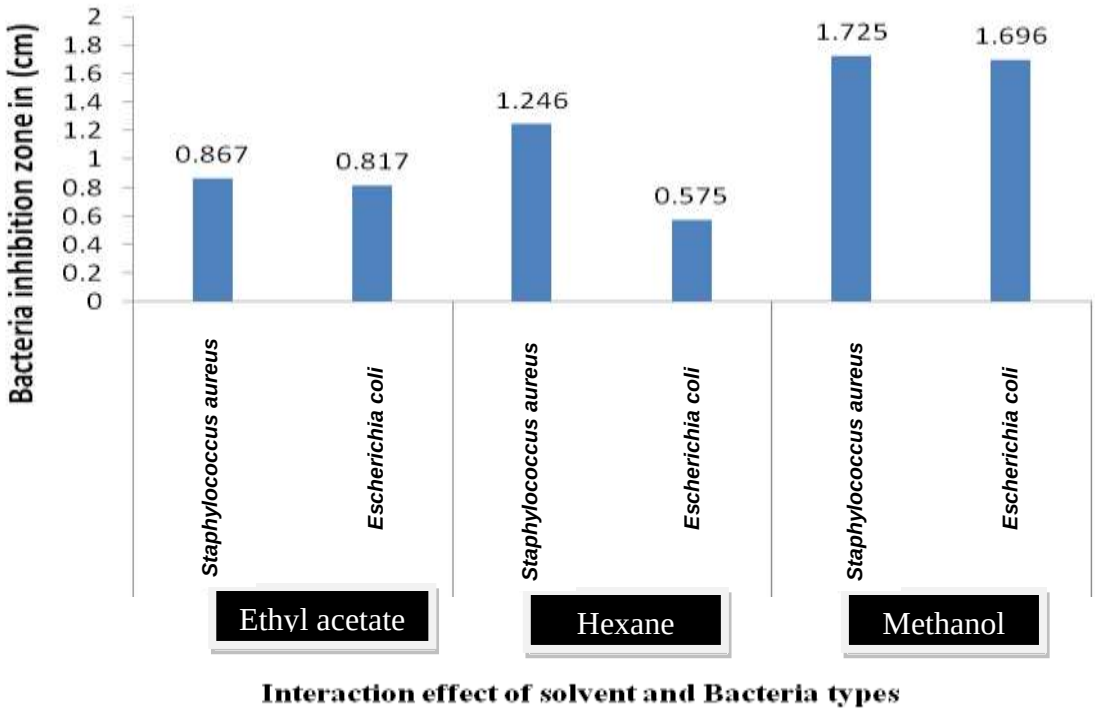

Figure 4. Interaction effects of solvent and Standardized Bacterial species types.

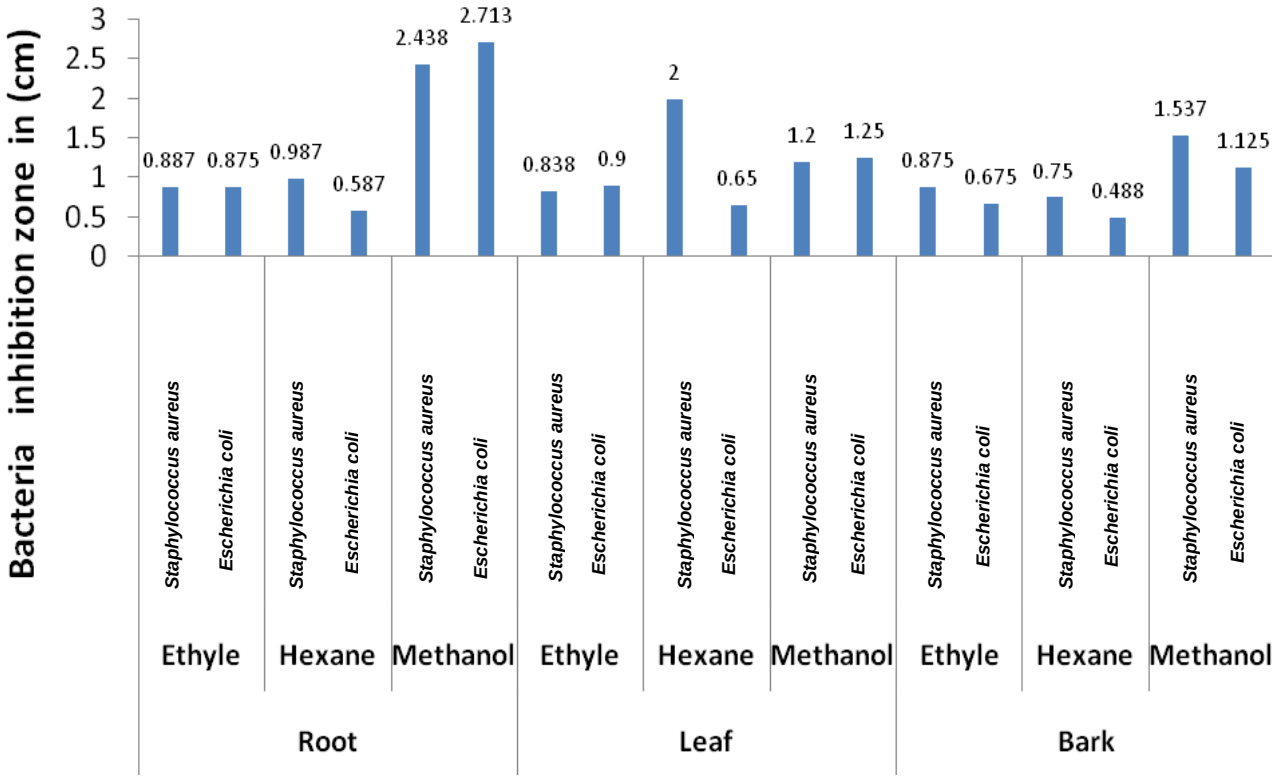

Figure 5. Interaction effects of parts of *H. abyssinica*, solvent, and bacteria types on growth inhibition zone.

using the different types of solvents, methanol exhibited relatively higher antibacterial activity on the selected study microorganisms followed by ethyl acetate and n-hexane. The oils extracted from root of *H. abyssinica* with all the three solvents have a better antibacterial activity as compared to other parts of the plant.

The oil extracts of *H. abyssinica* has a relatively better inhibitory effect on *S. aureus* than that of *E.coli.* The extracts of *H. abyssinica* from the three different parts were found active on both of the selected standard species of bacteria. This indicates that the oils have either cytotoxic or inhibitory effect and further investigations are therefore needed to clarify which compound is responsible for cytotoxic or inhibitory activities of the oils.

RECOMMENDATIONS

Data obtained from this research work put forward the following recommendations:

i) Chemical and pharmaceutical industries should work together with researchers and traditional medicinal plant collectors in order to utilize the maximum benefit of the plant under study.
ii) It is now understood in this research work that *H. abyssinica* oil has a remarkable cytotoxic effect on bacteria and further study is needed to indicate which compound inside the oils is responsible for the antibacterial property of the oil.
iii) Further researches should focus on the investigation of the antibacterial property of *H. abyssinica* parts and the minimum inhibitory concentration showing anti-bacterial activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abebe D, Ayehu A (1993). Medicinal plants and enigmatic health practices of Northern Ethiopia.

Akinyemi KO, Oluwa OK, Omomigbehin EO (2006). Antibacterial Activity of Crude Extracts Of Three Medicinal Plants Used In South-West Nigerian Folk Medicine On Some Food Borne Bacterial Pathogens African Journal of Traditional, Complementary and Alternative Medicines 3(4):13-22.

Biruktayet A, Gerhard G, Christine B (2010). Ethnomedicinal uses of *Hagenia abyssinica* (Bruce) J.F. Gmel. Among rural communities of Ethiopia. Journal of Ethnobiology and Ethnomedicine 6(20).

Bizuwerk A (2004). Multi-agent simulation for landscape management a case around Wondo Genet: Ethiopia. Centre for Geo-Information. Wageningen University 29 p.

Coyle MB (2005). Modes and Mechanism of antibacterial activity. Manual of Antibacterial Susceptibility Testing. American Society for Microbiology pp. 1-223.

Debela H, Zemede A, Ensermu K (2006). Use of traditional medicinal plants by people of 'Boosat' Sub District, Central Eastern Ethiopia. Ethiopian Journal of Health Science 16(2):141-155.

Desta B (1995). Ethiopian traditional herbal drugs. Part I: Studies on the toxicity and therapeutic activity of local taenicidal medications. Journal of Ethnopharmacology 45(1):27-33.

Endashaw B (2007). Study on Actual Situation of Medicinal Plants in Ethiopia. Prepared for JAICAF, Addis Ababa, Ethiopia.

Girma D (1998). Non-Wood Forest Products in Ethiopia. Data Collection and Analysis for Sustainable Forest Management in ACP Countries – Linking National and International Efforts, Addis Ababa, Ethiopia.

Hesham HAR, Abdurahman HN, Rosli MY (2016). Techniques for extraction of essential oils from plants: A Review. Australian Journal of Basic and Applied Sciences 10(16): 117-127

Hedberg O (1989). Flora of Ethiopia, Pittosporaceae to Araliaceae. In: Rosaceae. Hedberg, I. and Edwards, S. (eds). Addis Ababa University, Addis Ababa, pp. http://www.wgcfnr.org/resource/land.html. Wondo Genet College of Forestry and Natural Resources.

Legesse N (1995). Indigenous Trees of Ethiopia: Biology, uses and propagation techniques. Printed by the SLU Reprocentralen, Umeå, Sweden.

Lilian CN, Esther M, Charles M, Nicholas M, Christine B (2013). Antibacterial properties and toxicity of *Hagenia abyssinica* (Bruce) J.F. Gmel, Fuerstia africana T.C.E. Fries, Asparagus racemosus (Willd.) and Ekebergia capensis Sparrm. African Journal of Pharmacology and Therapeutics 2(3):76-82.

Ngeny L C, Magiri E, Mutai C, Mwikwabe N, Bii C (2013). Antibacterial properties and toxicity of *Hagenia abyssinica* (Bruce) J.F.Gmel, Fuerstia africana T.C.E. Fries, Asparagus racemosus (Willd.) and Ekebergia capensis Sparrm. African Journal of Pharmacology and Therapeutics 2(3):76-82.

Nibret E, Wink M (2010). Volatile components of four Ethiopian Artemisia species extracts and their in vitro antitrypanosomal and cytotoxic activities. Phytomedicine 17(5):369-374

Singh M, Govindarajan R, Nath V, Rawat AKS, Mehrotra S (2006). Antibacterial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. Journal of Ethnopharmacology 107:67-72.

Wolde T, Bizuayehu B, Hailemariam T, Tiruha K (2016). Phytochemical analysis and antibacterial activity of *Hagenia abyssinica*. Indian Journal of Pharmacy and Pharmacology 3(3):127-134.