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Research Article

Chemical composition and antimicrobial activities of leaves of sweet basil (*Ocimum basilicum L.*) herb

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ABSTRACT

Background: Leaves and flowering parts of *Ocimum basilicum* are believed to be rich of different phytochemicals and are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic, and tonic agents. Hence, the study was designed to evaluate the phytochemical constituents and antimicrobial activities of the leaves of sweet basil (*O. basilicum*) herb.
Methods: The leaf extract was used for screening of phytochemicals. A small amount of the powdered leaves of the plant was subjected to hydrodistillation to extract the essential oil, and the components of the essential oil were evaluated by gas chromatography-mass spectroscopy instrument. The extract was tested *in vitro* for its antibacterial activity against two bacteria; *Escherichia coli and Staphylococcus aureus* and antifungal activity against two fungi; *Aspergillus niger* and *Rhizoctonia bataticola* by paper disc diffusion method.
Results: Results revealed the presence of many phytochemicals such as alkaloids, tannins, flavonoids, cholesterol, terpernoids, glycosides, phenols, cardiac glycosides, carbohydrates, and phlobatannins. The essential oil extracted from the leaves of *O. basilicum* was found to have estragole (38.22%) as a major constituent followed by 1-isopropyl-4-methylenecyclohex-1-ene (11.10%). Tests of antimicrobial activity showed that the hydrodistilled oil was effective against all the tested bacterial and fungal strains. However, the crude extract was found not to have antimicrobial activity toward the tested bacteria and fungi.
Conclusion: So, the study has showed that the observed antimicrobial effect of *O. basilicum* essential oil on the bacterial and fungal isolate, though *in vitro* appear interesting and promising. So, emphasize have to made on the antimicrobial activities of the plant during the time of drug extraction.

Keywords: Antimicrobial activities, Essential oil and disc diffusion method, *Ocimum basilicum*

INTRODUCTION

Basil, or Sweet Basil, is a common name for the culinary herb *Ocimum basilicum* of the family Lamiaceae (mints), sometimes known as Saint Joseph's Worth in some English-speaking countries. Basil, originally from India, is a half-hardy annual plant, best known as a culinary herb prominently featured in Italian cuisine, and also plays a major role in the Northeast Asian cuisine of Taiwan, tropical part of Africa and the Southeast Asian cuisines of Indonesia, Thailand, Vietnam, Cambodia, and Laos. Depending on the species and cultivar, the leaves may taste somewhat such as anise, with a strong, pungent, and often sweet smell.[1]

The phytochemical evaluation of *O. basilicum L.* shows that it is rich in alkaloids, tannins, phytates, flavonoids and oligosaccharides.[2] In the eastern part of Ethiopia, the plant *O. basilicum L.* has been used for thousands of years as a culinary and medicinal herb. *O. basilicum L.* (sweet basil) is a cultivated plant which is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiatae and it as the most abundant of the genus *Ocimum*.[3] In the eastern part of Ethiopia, it is called "gosso-bilaa" or "Mesloba" (language of Oromiffa) while in Amharic it is named as "Beso Bla." Basil is cultivated commercially for its green, aromatic leaves, which are used fresh or dried as a flavoring or spice. The essential oil and oleoresin are extracted from the leaves and flowering tops via steam distillation/

hydrodistillation and used in place of the dried leaves for flavoring purposes.[4]

Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic, and tonic agents.[5] They have also been used as a folk remedy to treat various ailments such as; feverish illnesses, poor digestion, nausea, abdominal cramps, gastro-enteritis, migraine, insomnia, depression, gonorrhea, dysentery, and chronic diarrhea exhaustion. Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites, and skin infections.[6]

Basil is a very good source of beta-carotene. Beta-carotene helps to prevent damage to the cells by free radicals. Magnesium is essential mineral present in basil which helps the heart and blood vessels to relax, improving blood flow. Other nutrients found in basil include iron, calcium, potassium, and vitamin C.[7]

The basil comes in many different varieties, each with its own unique chemical composition and characteristic flavor. The flavor and character of any particular variety of basil is affected to a great extent by many external environmental factors, such as temperature, the type of soil, the geographic location, and even the amount of rainfall received by the individual plant. The seeds of sweet basil produce an aromatic essential oil which is prominent in the production of several drinks, cosmetics, and food of consumption. Methyl eugenol (78.02%), α-cubebene (6.17%), nerol (0.83%), α-muurolene (0.74%), 3, 7-dimethyloct-1, 5-dien-3,7-diol (0.33%), and β-cubebene (0.30%) were found as the major chemical constituents identified from *O. basilicum* essential oil. The essential oil from *O. basilicum* contained α-pinene, sabinene, β-pinene, myrcene, limonene, and (Z) - β-ocimene as the most important monoterpene hydrocarbons.[8]

Basil oil also has various chemical compounds that include camphene, cis-ocimene, camphor, linalool, methyl chavicol, γ-terpineol, citronellol, geraniol, methyl cinnamate and eugenol, and other terpenes.[9]

Knowledge of volatile components of Sweet basil is as important as its oil exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermides*, *Streptococcus pyogenes* as well as antifungal activities against *Aspargillus niger* and *Rhizocaticol bataticola* and also used for special flavor and cosmetics.[10]

So, there has been a research gap as there is no any work especially about isolation and characterization of the pure components of the crude extract from the leaves and antimicrobial activities of this herb in Ethiopia. So, since such medicinal herbs are widely distributed in different regions of Ethiopia and are traditionally used in the treatment of gonorrhea, dysentery, chronic diarrhea, wound healing, pimples, skin infections, and against different varieties of diseases, it was found to conduct the herb for chemical and antimicrobial investigation of the plant leaves.

It was also important to date on gas chromatography-mass spectroscopy (GC-MS) analysis of essential oil composition of Ethiopian sweet basils. Besides, the chemical composition of sweet basil, as well as its color, aroma and probably its medicinal characteristics, are changed according to the geographical zones and the season of the year.

METHODS

Description of the study area

Haramaya University is located at latitude of 9°20' North of the equator and 42°03' longitude east of the meridian. The university has a total area of about 46 km^2. It has a moderate average temperature of 16°C, and the mean maximum and minimum annual temperature are 24.02°C and 9.73°C, respectively. The mean annual rainfall is 780 mm. The 1980 m elevation of the area (*Weinadega*) ensures that it enjoys a relatively moderate and pleasant climate throughout the year.[11]

Apparatus and instruments

The apparatus and instruments used for the study were: separatory funnel, oven, filter paper (Whatman No. 1 filter paper), electric blender, pipettes (different size), water bath, beakers (different size), electronic balance, Clevenger's apparatus, flasks (different size), measuring cylinder, Rota vapor, polyethylene bag, ruler, pencil, spatula, shaker, distillation flask, heating mantle, petridish, refrigerator, holder, incubator, and GC-MS.

Chemicals, reagents and media

The chemicals and reagents used in the study were: distilled water, solvents (chloroform and methanol which were with analytical grades), anhydrous sodium sulfate, dimethylsulphoxide (DMSO), $FeCl_3$ (British Drug House Ltd., England), HCl (hydrochloric acid), H_2SO_4 (sulfuric acid), NaOH (sodium hydroxide), $CuSO_4$ (copper sulfate) (Baker Chemical Co., USA), NH_3 (ammonia), CH_3COOH (acetic acid), mollish reagent, ethanol, acetic anhydride (British Drug House Ltd., England), appropriate media for bacteria (Muller Hinton Agar [MHA], Blulux Laboratories (p) Ltd., India) and fungi (potato dextrose agar [PDA]), (OXOID Ltd., India), chloroamphenicol (Blulux Laboratory (p) Ltd., India), and Bavistin (Blulux Laboratories (p) Ltd., India and Ltd., UK).

Collection of the plant materials

The leaves of *O. basilicum* (sweet basil) were collected from farmland and local markets at Bati, Harergea zone, near

Haramaya University, Ethiopia. After collection, the leaves were washed repeatedly first with tap water and then with distilled water and were allowed to air dry completely for 72 hrs at room temperature.

Solvent extraction of the plant material

1 kg of the leaves of the plant was air dried and ground using an electric blender. The resulting powder was stored in a polyethylene bag to avoid it from an attack of certain environmental conditions (moisture, air, and other surrounding dust). The sample of powdered leaves (200 g) was first soaked with 600 ml of chloroform and methanol (1:1) for 48 hrs with continuous stirring at room temperature. Then the extracts were filtered using Whatman No. 1 filter paper. Finally, the filtrates collected as such were concentrated at 40°C by Rota vapor under reduced pressure, and the crude extract was stored in a refrigerator at 4°C for further analysis.

Phytochemical analysis

The crude extract was used for screening of phytochemicals such as alkaloids, proteins, flavonoids, glycosides, carbohydrates, terpinoids, and tannis. Preliminary phytochemcial screening was carried out on the plant crude extract, according to the standard procedures described by.[12-15]

Extraction of essential oil by hydrodistillation

60 g of the powdered leaves of *O. basilicum L.* was placed in 1000 ml round bottom flask. Then 500 ml of distilled water was added and mixed thoroughly. The flask was fitted with Clevenger's apparatus, a glass condenser and heated using heating mantle and hydrodistilled at atmospheric pressure for 3 hrs. The oil was separated from the aqueous layer by adding 100 ml of chloroform in a separatory funnel. The small amount of aqueous liquid left with the chloroform was then dried by adding 5 g of anhydrous sodium sulfate and filtered using Whatman No. 1 filter paper. Finally, the mixture was concentrated using a rotary evaporator, and this was kept in the refrigerator. The chemical constituents of the oils were determined by GC-MS at Ambo University, Ethiopia.

GC-MS analysis of the essential oil of O. basilicum

A GC-MS instrument from Agilent Technologies (Santa Clara, CA, USA) equipped with a 6890N network GC system, 5975 inert mass selective detector, 7683B series autosampler injector (10 µl in size), G1701DA GC/MSD Chem Station and HP5MS column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) coated with 5% phenyl 95% methyl poly siloxane was used for analyzing the samples. 2 µl essential oil solutions in chloroform was injected through autosampler and analyzed with HP5MS column.

Column temperature was programed as follows: 55°C to 120°C at 20°C/min, 120°C to 150°C at 1.5°C/min, 150°C to 250°C at 20°C/min, 250°C (10 min) and 3 min solvent delay. The mass spectra transfer line temperature was 280°C. The carrier gas was helium (1 ml/min) with a split ratio equal to 100:1. Injector, quadruple and detector temperatures were 220, 150, and 250°C, respectively. The mass spectra were recorded in electron ionization mode at 70 eV with scanning from 50 to 500 amu (atomic mass unit) at 0.5 sec with the mass source being set at 230°C. The identification of the compounds was based on retention time (tR), mass fragmentation pattern and by comparison with the spectral data available in the literature. Integration of peaks was performed using Hewlett Packard Chem Station software (G1701BA Version B.01.00) for quantification of the peaks.

Antimicrobial assay

All the extracted compounds were tested *in vitro* for their antibacterial activities against two bacteria; *E. coli and S. aureus* and antifungal activity against two fungi; *A. niger* and *R. bataticola* by paper disc diffusion method. For bactericidal and fungicidal studies, MHA and PDA media, respectively, were used. Known antibiotic such as chloramphenicol was used as a standard drug (as reference) in bactericidal and Bavistin was used as a standard drug against fungi. From inhibition zone data, correlations of structures with antibacterial activities compounds were critically examined.

The test bacterial strains, *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) were transferred from the stock cultures and streaked on Mueller Hinton plates and incubated for 24 hrs at 37°C. Well separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological/ inoculating loop to autoclaved Mueller Hinton agar that was cooled to about 45°C in a water bath and mixed by gently swirling the flasks. The medium was then poured into sterile Petri dishes, allowed to solidify and used for the biotest.[16]

For the test of fungi, mycelia plugs from stock cultures were transferred to PDA plates and incubated for 7 days. Then spores of *A. niger* were harvested by washing the surface of the colony using 10 ml sterile distilled water and transferred to 250 ml autoclaved PDA cooled to about 45°C in a water bath. Likewise, mycelium of *R. bataticola* were washed with 10 ml sterile distilled water, macerated in a blender and the mycelia suspension was transferred to 250 ml autoclaved PDA cooled to about 45°C in a water bath. The medium containing spore or mycelia suspension was poured to sterile plates allowed to solidify and was used for the disc diffusion bioassay.[16]

Paper discs of about 6 mm in diameter were cut from Whatman No. 1 filter paper with an office paper punch and placed in a beaker covered with aluminum foil and sterilized in an oven at 180°C for 1hr. Then 10 µl and 20 µl of solution of compounds were pipetted to the discs in two

replications. After allowing the solvent to evaporate, the paper discs impregnated with the sample solutions were then transferred with sterile forceps to PDA seeded with spore suspension of test fungi as described above. The Petri dishes were incubated at 26°C for 7 days. The entire test was performed in duplicate. The antifungal activity was evaluated by measuring the zone of inhibition against the test organism and DMSO as a positive control. Similar procedures were followed in antibacterial activities test except the paper discs were transferred to MHA plate seeded with bacteria and incubated at 37°C for 24 hrs.

RESULTS

Yield of the crude extract and the essential oil

The crude extract was dark greenish in color with a yield of 23.5 g (11.75% w/w). On the other hand, the hydrodistillation of the air dried powdered leaves of the plant yielded pale yellowish essential oil of 2.18 g (3.63% w/w).

Phytochemical screening

The phytochemical analysis of the crude extract revealed the presence of alkaloids, tannins, flavonoids, cholesterol, terpernoids, glycosides, cardiac glycosides, phenols, carbohydrates, and phlobatannins and absence of saponins and proteins as shown in Table 1.

GC-MS characterization of O. basilicum L. essential oil

GC-MS analysis of the essential oil (Figure 1) has shown the presence of 34 components and 15 of these compounds (Table 2) were identified by means of their tR, mass spectral fragmentation patterns and by comparing their mass spectra with the NIST 2005 library of mass spectra. Unidentified components were present in such low amounts that either no mass spectrum could be recorded, or the spectrum was too poor for interpretation.

Table 1: Phytochemical constituents of the leaves of *O. basilicum*.

Constituents	Results
Alkaloids	+
Carbohydrates	+
Cardiac glycosides	+
Cholesterol	+
Flavonoids	+
Glycosides	+
Phenols	+
Phlobatannins	+
Proteins	-
Saponins	-
Tannins	+
Terpinoids	+

+: Present, -: Not present, *O. basilicum: Ocimum basilicum*

Antimicrobial effects

The antimicrobial activities of *O. basilicum* (chloroform/methanol (1:1)) crude extract and essential oil against the microorganisms were examined in the present study, and their potency, were assessed by the presence or absence of inhibition zones and zone diameter compared with some antibiotics. Results are given in Tables 3 and 4.

The results showed that the essential oil was effective to kill bacteria and fungi, and the two different concentrations of

Table 2: GC-MS analysis of the leaf extract of *O. basilicum* essential oil.

tR	Compound name	Relative percentage
7.29	Eucalyptol	3.462
8.771	L-Fenchone	5.793
9.121	1-isopropyl-4-methylenecyclohex-1-ene	11.104
11.142	α-Pinene	3.813
11.872	Estragole	38.226
13.213	p-mentha-1(7), 8-diene	6.012
14.52	Methyl-2-phenyl-prop-2-enolate	1.887
15.859	Eugenol	1.531
16.569	(E)-methyl cinnamate	6.510
17.389	Isocaryophillene	2.460
17.75	trans-α-Bergamotene	1.268
18.27	Eudesma-3,7(11)-diene	3.164
18.89	α-Cubebene	2.474
19.52	beta-bisabolene	2.395
20.331	α-Caryophyllene	4.569
Total		94.67

tR: Retention time, GC-MS: Gas chromatography-mass spectroscopy, *O. basilicum: Ocimum basilicum*

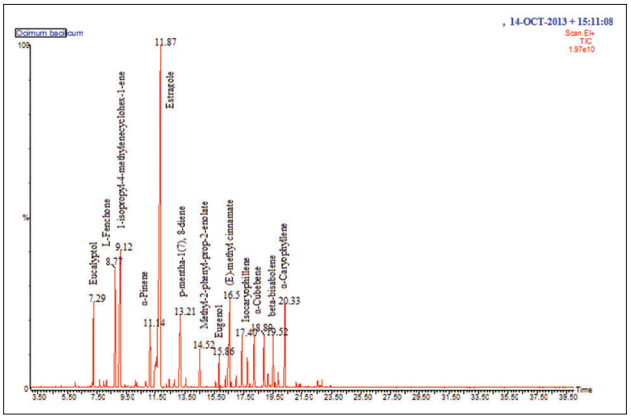

Figure 1: Typical gas chromatogram of *Ocimum basilicum* essential oil.

Table 3: Zone of fungal growth inhibition (mm) of chloroform/methanol (1:1) crude extract and essential oil of *O. basilicum*.

Sample	Fungi		
	Dose (µl)	*A. niger*	*R. bataticola*
Oil	10	7.50±0.71	8.20±0.28
	20	8.00±0.71	12.75±0.35
Crude extract	10		-
	20		-
Bavistin	10	18.50±0.71	21.50±0.71
	20	19.50±0.71	22.50±0.71
DMSO	10		-
	20		

Values are represented in terms of mean of the two trials±SD; -: Stands for no inhibition, SD: Standard deviation, *O. basilicum: Ocimum basilicum, A. niger: Aspergillus niger, R. bataticola: Rhizoctonia bataticola*, DMSO: Dimethylsulphoxide

Table 4: Zone of bacterial growth inhibition (mm) of chloroform/methanol (1:1) crude extract and essential oil of *O. basilicum*.

Sample	Bacteria		
	Dose (µl)	*E. coli*	*S. aureus*
Oil	10	8.50±0.71	8.50±0.71
	20	8.80±0.56	8.75±0.35
Crude extract	10		-
	20		-
Chloroamphinicol	10	19.50±0.71	23.25±0.35
	20	33.50±0.71	28.75±0.35
DMSO	10		-
	20		-

-: Stands for no inhibition, *O. basilicum: Ocimum basilicum*, DMSO: Dimethylsulphoxide, *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus*

the oil have different inhibition zones (Figure 2). Lack of antimicrobial activity of the crude extract may be due to the low concentration of the sample used.

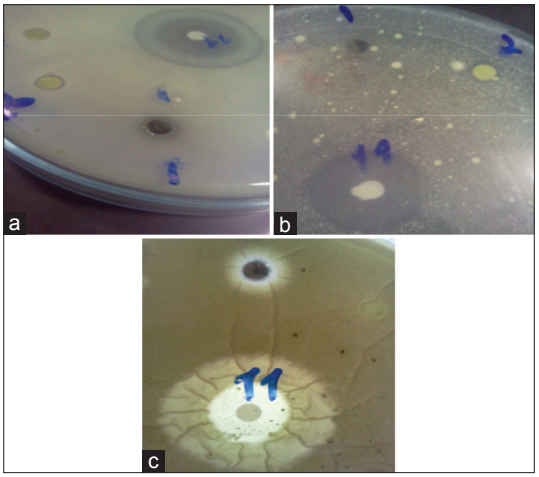

Figure 2: Inhibition zone of *Ocimum basilicum* leaves oil against, (a) *Escherichia coli, (b) Staphylococcus aureus,* and (c) *Aspergillus Niger.* Where, 1=Inhibition zone of oil, 2=Crude extract, 11=Control drug.

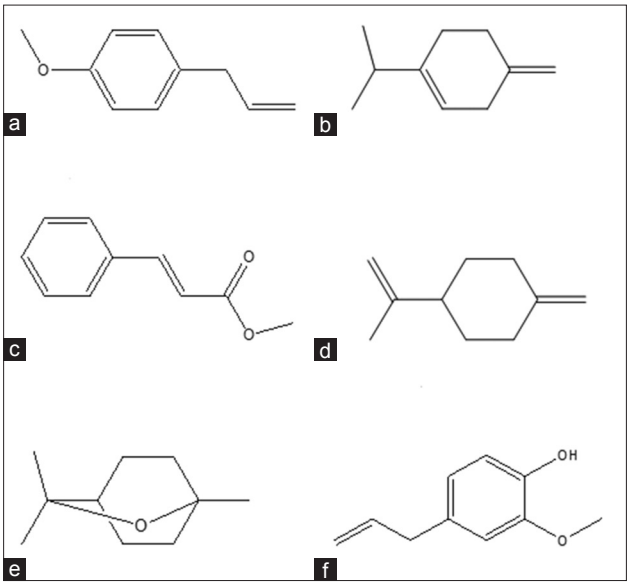

Figure 3: Proposed structures of some of the major components of *Ocimum basilicum* essential oil. (a) Estragol, (b) 1-isopropyl-4-methylenecyclohex-1-ene, (c) (E)-methyl cinnamate, (d) p-mentha-1(7),8-diene, (e) Eucalyptol, and (f) Eugenol.

DISCUSSION

The findings of this study on the phytochemical screening of *O. basilicum* were agreed with[17] who reported that the leaves of *O. basilicum* are rich in Tannins, flavonoids, cholesterol, terpernoids, glycosides, cardiac glycosides, and phlobatannins which were also similar with those clarified in this research. The leaf extracts of *O. basilicum* are rich in various phytochemicals and now a day's these compounds are used tremendously to synthesize drugs for pharmacological actions and are substantial for different foodstuffs.[18]

The GC-MS spectrum of the essential oil and structures of some of its major components are shown in Figures 1 and 3, respectively, and the essential oil components identified from the leaves of Sweet basil are shown in Table 2. A total of 15 compounds representing 94.67% of Sweet basil oil were identified. Estragole (38.226%), 1-isopropyl-4-methylenecyclohex-1-ene (11.104%), 2-propenoic acid-3-phenyl-methyl ester ((E)-methyl cinnamate) (6.510%), p-mentha-1(7), 8-diene (6.012%), L-Fenchone (5.793%), α-caryophyllene (4.569%), eucalyptol (3.462%), α-pinene (3.813%), α-cubebene (2.474%), isocaryophillene (2.460%), methyl-2-phenyl-prop-2-enolate (1.887%), eugenol (1.531%), eudesma-3,7(11)-diene (3.164%), beta-bisabolene (2.395%), and trans-α-bergamotene (1.268%) were found as the major compounds. According to the GC-MS display,

nine monoterpenes (eucalyptol, L-Fenchone, 1-isopropyl-4-methylenecyclohex-1-ene, α-pinene, estragole, p-mentha-1(7),8-diene, methyl-2-phenyl-prop-2-enolate, eugenol, and (E)-methyl cinnamate) and six sesquiterpenes (isocaryophillene, trans-α-bergamotene, eudesma-3,7(11)-diene, α-cubebene, beta-bisabolene, and α-caryophyllene) were identified in the essential oil.

The oil of *Ocimum* species was the subject of former studies.[19-23] It was previously reported that the oil of *O. basilicum* contained linalool (69%) and eugenol (10%).[23] Linalool (45.7%) and eugenol (13.4%) were reported to be the main components of the previously analyzed materials.[19] However, in this research estragole (38.226%), 1-isopropyl-4-methylenecyclohex-1-ene (11.104%), ((E)-methyl cinnamate) (6.510%), p-mentha-1(7), 8-diene (6.012%), L-Fenchone (5.793%), α-caryophyllene (4.569%), and α-pinene (3.813%) were found as major components of the essential oil of the leaves of *O. basilicum* having higher percentages. This significant difference is due to the fact that the chemical compounds of any plant essential oil can vary greatly depending on geographical region, the age of the plant, local climate; seasonal variations, experimental conditions, and genetic difference are responsible for the changes in the types of chemical compounds.

The results of this study are also in a good agreement to those of[24] who reported the oxygenated monoterpenes as the major compounds in Turkish *O. basilicum* essential oil. According to;[8] the main compounds of oil from basil herb collected in Turkey were eugenol (78.02%), α-cubebene (6.17%) which were also reported in this research as the components of the essential oil of the leaves of *O. basilicum*. Estragole, which hold the highest composition of the essential oil in this research was also reported as the major component of the oil, extracted from the leaves of *Ocimum* species. α-cubebene, α-caryophyllene, and L-Fenchone were also identified in the areal parts of *O. basilicum*.[25]

The essential oil suspended with 10 µl and 20 µl of DMSO exhibited inhibition effects against the two fungal strains; namely *A. niger* and *R. bataticola*. On the other hand, the crude extract did not show any inhibition zone against the two fungi. The strongest inhibition activity of sweet basil oil was observed against *R. bataticola* (8.20 mm and 12.75 mm). Besides, the inhibition zone of the control drug (Bavistin) was higher in *R. bataticola* than *A. Niger* at both doses.

The essential oil also showed antibacterial activity against the two test bacteria (*E. coli and S. aureus*). Inhibition zones of the oil were to a little extent higher in *E. coli* than in *S. aureus*, hence, the oil has a stronger antibacterial activity toward *E. coli* than *S. aureus*. The standard samples showed the greatest inhibition while the negative control solvent (DMSO) has no inhibition zone.

Previous works reported by different researchers also showed that the essential oils extracted from *O. basilicum* possess an excellent antimicrobial activity toward different microorganisms including Gram-positive and Gram-negative bacteria and fungi. Sweet basil essential oils exhibited good antimicrobial activity against a wide range of microorganisms.[26] *O. basilicum* essential oil showed moderate antibacterial activity.[27] Whereas,[28,29] described that the Gram-positive strains of bacteria showed higher sensitivity to *O. basilicum* essential oils than those of their counterpart. Besides,[28,30] also reported the antifungal activity of essential oils from *O. basilicum* and its main component, linalool.

The antimicrobial activities of essential oils from *O. basilicum* may be due to partly the presence of high content of linalool.[31] Therefore, *O. basilicum* essential oils are valuable not only as foodstuffs but also it could replace synthetic antimicrobial agents in the future. Studies should also be extended to evaluating the practical effectiveness of essential oil against the growth of different food borne and spoiling microbes under the specific environmental, storage, and food processing conditions.

CONCLUSION

From this study, it was observed that the hydrodistillation of the leaves of *O. basilicum* provided an aromatic essential oil of a better yield. The chloroform/methanol (1:1) extracts exhibited high inhibitory activity on the test organisms. This can be deduced to the ability of Chloroform or Methanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antimicrobial activity on the test organisms. This study, however, can justify the use of the plant in traditional medicine practice as a therapeutic agent and can explain the long history use of these plants. So, emphasize have to make on the antimicrobial activities of the plant during the time of drug extraction.

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