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RESEARCH ARTICLE OPEN ACCESS

Antimicrobial Activity and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Saudi Arabian *Ocimum basilicum* Leaves Extracts

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Abstract

The present study evaluates the antimicrobial activity of *Ocimum basilicum* leaves extracts using well diffusion assay. Microbicidal or Microbiostatic activities were determined using (MBC or MFC) /MIC ratio. All *O. basilicum* extracts ethanol, methanol, and water possess antimicrobial activity. Methanol was the best solvent with greater inhibitory activity followed by ethanol then water against bacteria. However, all three solvents showed no difference in their inhibitory activity against yeast. The MIC and MBC values were in decreasing order methanol, ethanol then water against bacteria whereas *Candida albicans* was more sensitive at MIC 12.5µg/mL than *C. tropicalis* at MIC 25µg/mL and MFC values were lower against *C. albicans* than *C. tropicalis* at 25µg/mL and 50µg/mL, respectively in all type of solvents. The ratio of (MBC or MFC) /MIC were one to three-folds. The GC-MS result showed the presence of several important chemical compounds like terpene, steroids, phenols, esters, and fatty acids most of these compounds were reported to have an antimicrobial activity. The study indicates the importance of *O. basilicum* extracts as microbicidal agent with wide spectrum and high inhibitory properties in low concentrations. Therefore, *O. basilicum* leaves extracts may be important in the field of antimicrobial production as alternatives to antibiotics.

Keywords: Antimicrobial activity, GC-MS Analysis, *Ocimum basilicum*, Antibiotics.

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INTRODUCTION

The discovery of antibiotics in the mid-nineties appears to have solved most of the common problems in microbial infections. But the risk lies in the pathological cases in which the microbes are resistant to antibiotics or develop antibiotic resistance[1,2]. The massive use of antibiotics in the past 80 years both appropriate and inappropriate has led to fast antibiotic resistance. Bacteria are able to resist various antibiotics through horizontal gene-transfer or mutation[3]. Furthermore, other bacteria are intrinsically resistant to antibiotic, for example not having antibiotics target sites or having low permeability to antibiotics[4,5]. The resistance mechanisms for antibiotics occur by enzymatic inactivation, site target modification, active elimination, or absorption inhibition[6]. Estimated report indicate that the resistance of bacteria to antibiotics and their loss of ability to treat many bacterial infections will be the first killer in year 2050, where deaths are expected to reach 10 million per year, which means they are higher than deaths due to cancer[7].

In fact antibiotic resistance has become the third threat to public health in the 21st century[8]. Moreover, antimicrobial resistance causes high economic burden including high therapeutic expenses and longer hospital stay[9,10]. Accordingly, it is necessary to develop anti-microbial agents as an alternative to treat infectious diseases. Several studies showed that plants substances are a promising source for the discovery of novel antimicrobial agents[11-13].

Lamiaceae is one of most famous medicinal family plants, contains 236 genera and about 6000 species[14]. *Ocimum* sp. is one of the important geneses in this family; it contains about 150 species that greatly differ in their morphology, aromatic composition, essential oil content, and chemical composition[15]. *Ocimum basilicum* is an annual herb grows in different regions of the world[16] and is known as Basil or Sweet Basil also named as king of the herbs[17,18]. This aromatic plant traditionally used as fresh or dried leaves as food flavoring, food preservative, medicinal plant and perfumery[19-21]. Several studies reported that Sweet Basil leave extract were able to cure various illnesses and symptoms[22,23]. Forthermore, Sweet Basil has activity against inflammation, viruses, bacteria and fungi[24-26]. It has pharmacological effects against several diseases, with great antioxidant, anticancer and anti-aging properties[27,28]. Moreover, basil leaves essential oils and extracts were found to be a rich source of phytochemical compounds such as chavicol, linaloon, methyl ether, estagole, eugenol, methyl eugenol, and methyl chavicol[29-31].

There are no previous studies involving this microbial group in a single study using alcoholic and aqueous *O. basilicum* extracts and the antimicrobial efficiency (Microbicidal or Microbiostatic). It is also noteworthy that most previous studies have highlighted the study of essential oil of O. *basilicum* and their anti-microbial activity while the O. *basilicum* extract received less attention. Therefore, the aim of this study was to compare the antimicrobial activity of three solvents of O. *basilicum* leaves extracts; ethanol, methanol and water, determine the antimicrobial efficiency and their phytochemical components.

MATERIALS AND METHODS

Plant material

Ocimum basilicum L. (sweet basil) plant were purchased from a vegetable market in Dammam, Saudi Arabia. The plant was identified by Department of Biology, College of science, Imam Abdulrahman Bin Faisal University using The Herb Society of America[32].

Preparation of plant extracts

Plant leaves were washed thoroughly with tap water and dried at room temperature. Dried leaves were ground to fine powder using a blender. Sixty grams of the powder plant was transferred one by one to three Erlenmeyer flasks containing 300 mL of distilled water, ethanol (80%), and methanol (80%) to obtain a final concentration of 20% g/mL. The mixtures were placed in a shaker at 300 rpm/min for 72 hours at 20°C to allow extraction of active compounds. The extract solutions were filtered with Whatman No. 1 filter paper then bacterial filters and filtrates were concentrated using an oven at 80°C. Dimethyl Sulfoxide (DMSO) was used to re-suspend the residues to a final concentration of 20% and the flasks were sealed and kept at 4°C for further use[33].

Test microorganisms

Eight microorganisms were used three Gram positive bacteria (*Staphylococcus aureus*

ATCC24213, *S. aureus* and *Bacillus subtilis*) three Gram negative bacteria *(Escherichia coli* ATCC25922, *E. coli and Pseudomonas aeruginosa*) and two yeasts (*Candida albicans* and *Candida tropicali*). Most microorganisms were obtained from King Fahd Hospital, AlKhobar, Saudi Arabia. Except *B. subtilis* was obtained from the Biology Department, College of Science, Imam Abdulrahman Bin Faisal University.

Agar well diffusion technique

Antimicrobial activity was carried out using agar well diffusion technique[34]. One mL of microorganisms cultures age 18-24 h (standard inoculums 1-2x10^8 CFU/mL 0.5 McFarland standard) were transfer to Petri plates, and 15 mL of nutrient agar was poured into the plates. After the cultures solidified, wells sizes 5 mm were punched using a sterile cork borer. Each well was filled with 50 µL of plant extracts; DMSO was used as a negative control, erythromycin (E15 mcg) as a positive control for the bacteria and nystatin (100mg) as a positive control for the yeasts. Treated plates were placed in a refrigerator for about one hour to allow diffusion of the plant extract and controls. Then plates were incubated for 48 hours at 37°C. Antimicrobial activity was recorded in millimeters by measuring the zones of inhibition around the wells. All experiments were performed with five replicates.

Determination of Minimum Inhibitory Concentration (MIC)

Method of Omura *et al.*[35] was preformed to determine the Minimum Inhibitory Concentration (MIC) of the plant extracts. Two- fold dilution of the plant extracts was made with nutrient broth media using 96 well microtitre plates. Standard bacterial inoculums at the concentration of 1-2x10^8 CFU/mL were added to the wells to a final concentration of 50%. Well number 11 left as positive control contain growth media and bacterial inoculum and well number 12 left as negative control contain growth media and plant extract. The MICs were read after overnight incubation at 37°C using a microtitre plate's reader at a wavelength of 630 nm. All experiments were performed in three replicate.

Determination of Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

Pour plate method was used to determine the MBC and MFC[36]. From the MIC experiment concentrations that showed no bacterial growth were sub-cultured using Petri plates, and then 12 mL of melted nutrient agar media was poured over it and gently mixed and left to solidify. The inoculated plates were incubated at 37°C for 48 hours. The lowest concentration that showed no visible colonies were recorded as MBC. All experiments were performed in three replicate.

Determination of antimicrobial efficiency (Microbicidal or Microbiostatic)

The antimicrobial efficiency of the of *O. basilicum* extracts (ethanol, methanol and water) was determine by using the ratio of MBC or MFC/MIC[37].

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometer Model QP2010 SE (Shimadzu-Japan) with 5 Sil MS 5% diphenyl/95% dimethyl polysiloxane capillary column (30 meter, 0.25 mmID, 0.25-µm df) was used to screen the bioactive compounds of Sweet Basil plant extracts. Hundred micromilliliters of plant sample were diluted using 1400µl of DMSO. One µL of diluted sample (100/1400, V/V in DMSO) was injected in the split mode with a split ratio 1:10. For GC-MS detection, electron impact ionization system with ionization energy of 70eV was used. Carrier gas was pure helium (99.999%) at a constant column flow 0.7ml/min and total flow was 10.4 ml/min. Flow control mode was linear velocity of 29.6cm/sec. Injector temperature was set at 250°C and ion-source temperature 250°C. The oven temperature was programmed from 50°C to 300°C, hold time was 3 min, and total run time was 29 min. ACQ Mode Scan range from 35 m/z to 500 m/z with scan speed 2500. Chemical compounds were identified by National Institute of Standards and Technology (NIST 08) library match.

Statistical analysis

The antimicrobial activities of *O. basilicum* leaves extracts between the solvents and the microbes were analyzed using SPSS Version 23.0[38] at significance p <0.01.

RESULTS

The antimicrobial activity of *O. basilicum* extracts (ethanol, methanol and water) was tested through the presence or absence of clear growth zones around the well. The results clarify that

solvent type affect the inhibitory activity (Table 1). In general, the methanol extract showed the highest inhibitory activity against Gram positive bacteria and Gram negative bacteria follow by ethanol extract then water extract. However, all solvents were equal in their impact on the yeasts. The results showed that *S. aureus* and *E. coli* were resistant to the Erythromycin (E15 mcg) antibiotic

Table 1. Antimicrobial activity of *O. basilicum* leaves extracts at concentration of 20% g/mL using agar well diffusion technique

Micro-organisms	Ethanol extract	Methanol extract	Water extract	Significance ($p \leq 0.01$)*	Positive control**	Negative control***
			Gram positive bacteria			
S. aureus ATCC24213	10.7±0.6	12.4±0.6	7.5± 0.9	0.001	14±1.0	R
S. aureus	11.5±0.5	11.8±0.3	11.3±0.3	0.003	R	R
B. subtilis	10.7±0.6	13.7±0.6	10.5±0.5	0.001	20±1.0	R
			Gram negative bacteria			
E. coli ATCC25922	11.5±0.3	12.3±0.6	10.8±0.3	0.001	9.0±1.0	R
E. coli	11.8±0.3	12.8±0.3	11.3±0.3	0.001	R	R
P. aeruginosa	11.8±0.3	12.3±0.3	11.4±0.5	0.002	10.8±0.3	R
			Yeasts			
C. albicans	12.6±0.3	12.5±0.6	12.6±0.5	0.1	19.2±0.8	R
C. tropicalis	11.8±0.3	11.9±0.3	11.7±0.5	0.006	17.3±1.0	R
*Significance($p \leq 0.01$)	0.00	0.002	0.00	-	-	-

* *P*-values are less than 0.01 which means that there is a significant difference between the tested microbes or between the used solvents.
**Erythromycin (E15 mcg) positive control for the bacteria and Nystatin (100mg) positive control for the yeasts. ***DMSO, negative control.

Table 2. Determination of Minimal Inhibitory Concentration (MIC) µg/ml, Minimal Bactericidal Concentration (MBC), Minimal Fungicidal Concentration (MFC) µg/ml and their ratio of *O. basilicum* extracts

Micro-organisms	MIC, MBC and MFC (µg/ml) of Sweet Basil extract								
	Ethanol extract			Methanol extract			Water extract		
	MIC	MBC or MFC	Ratio*	MIC	MBC or MFC	ratio	MIC	MBC or MFC	Ratio
				Gram positive bacteria					
S. aureus ATCC24213	12.5	50	2	12.5	25	1	25	50	1
S. aureus	12.5	50	2	12.5	25	1	25	100	2
B. subtilis	6.25	12.5	1	3.125	6.25	1	25	50	1
				Gram negative bacteria					
E. coli ATCC25922	12.5	50	2	12.5	25	1	25	50	1
E. coli	12.5	100	3	12.5	50	1	25	100	2
P. aeruginosa	12.5	50	2	12.5	25	1	25	50	1
				Yeasts					
C. albicans	12.5	25	1	12.5	25	1	12.5	25	1
C. tropicalis	25	50	1	25	50	1	25	50	1

Ratio* (MBC or MFC)/MIC

Table 3. GC-MS analysis of *O. basilicum* extracts and their biological activities

No	Compound name	Peak Area% EL	ML	WL	Molecular Formula	Compound Nature and Biological activities
1	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	19.26	10.47	1.28	$C_{18}H_{30}O_2$	Linolenic Omega-3 polyunsaturated fatty acid, Anti-inflammatory, Cancer preventive, Antieczemic and Nematicide[39].
2	n-Hexadecanoic acid	9.68	3.54	1.14	$C_{16}H_{32}O_2$	Palmitic saturated Fatty acid ester, Antioxidant, Nematicide, Hypochloesterolemi and Antiandrogenic, pesticide[39, 40].
3	9,12,15-Octadecatrienoic acid, ethyl ester, (Z	8.59	3.81	0.3	$C_{20}H_{34}O_2$	Linolenic acid, ethyl este No activity was reported.
4	Phytol	4.96	1.17	0.2	$C_{20}H_{44}O$	Diterpene, Antimicrobial, Cancer preventive, Anticancer and Anti-inflammatory[39, 40].
5	Phytol, acetate	2.69	0.5	0.27	$C_{22}H_{42}O_2$	Anti-inflammatory, antileishmanial and antitrypanosomal[41].
6	Methyleugenol	2.42	2.25	0.3	$C_{11}H_{14}O_2$	Phenolic compounds, Anti-inflammatory, Antioxidant, Antimicrobial, Nematicide and insecticide[42].
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.04	0.81	0.27	$C_{20}H_{40}O$	Terpene Alcohol, Antimicrobial, Antioxidant, Anti–inflammatory and flavoring agent[39,43,44].
8	Eugenol	0.67	0.72	0.19	$C_{10}H_{12}O_2$	Phenolic compounds, Antimicrobial, Nematicide, insecticide and food additive[42, 45].
9	Hexadecanoic acid, methyl ester	0.32	2.56	0.2	$C_{17}H_{34}O_2$	Palmitic saturated fatty acids ester, Antioxidant, Flavor, Hypocholesterolemic Pesticide and 5-Alpha reductase inhibitor[39].
10	S-Methyl methanethiosulphonate	0.28	0.21	1.03	$CH_3SO_2SCH_3$	Ester and Antimutagenic agent[46].
11	Benzeneacetic acid	0.15	0.27	0.78	$C_9H_{10}BrNO_2$	No activity was reported.
12	Vitamin E	0.79	0.29	-	$C_{29}H_{50}O_2$	Lapid, Anti-Alzheimer, Antiaging, Antioxidant and Anti–inflammatory[41,47,48].
13	Resorcinol	0.21	0.13	-	$C_6H_6O_2$	1,3-isomer of dihydroxyphenol, not a bacterial mutagen[49,50].
14	Propane, 3-chloro-1,1,1-trifluoro	19.84	-	4.26	$C_3H_4ClF_3$	No activity was reported.
15	Hexadecanoic acid, ethyl ester	4.9	-	0.22	$C_{18}H_{36}O_2$	Palmitic saturated fatty acids, Antioxidant, Flavor, Anti-androgenic, Nematicide, Hemolytic and Hypocholesterolemic[39].
16	9,12-Octadecadienoic acid (Z,Z)-	0.11	-	0.22	$C_{18}H_{32}O_2$	Article I. Linoleic acid polyunsaturated fatty acid, Anti-inflammatory, Anticancer, Antiacne and Nematicide[39].

17	Benzoic acid, 4-hydroxy-	2.05	-	-	$C_7H_6O_3$	Phenolic compounds and No activity reported[51].
18	Salicylic acid	0.12	-	-	$C_7H_6O_3$	Phenolic compounds, Bacteriostatic, Fungicidal, Anti-inflammatory and Antibacterial[52].
19	4,5-Dichloro-1,3-dioxolan-2-one	-	24.23	49.87	$C_3H_2Cl_2O_3$	No activity reported
20	gamma-Sitosterol	-	11.52	4.19	$C_{29}H_{50}O$	Steroid and Antidiabetic drug[53].
21	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	-	6.3	0.71	$C_{19}H_{32}O_2$	Linolenic polyunsaturated fatty acid methyl ester, Antimicrobial, Antiarthritic, Anticancer and Antiasthama[39].
22	stigmasterol	-	4.94	0.78	$C_{29}H_{48}O$	Steroid, Antioxidant, Antimicrobial, Anticancer, Antiarthritic, Antiasthma, Anti inflammatory and diuretic[40,54].
23	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	-	2.12	0.13	$C_{19}H_{34}O_2$	Methyl linoleate, Analgesic, Anti-inflammatory and Ulcerogenic[55].
24	Octadecanoic acid		0.67	0.45	$C_{17}H_{35}CO_2H$	Stearic saturated fatty acid No activity reported
25	Lupeol	-	0.23	0.61	$C_{30}H_{50}O$	Article II. Triterpenoid, Antimicrobial, Anti-inflammatory, Anticancer, and Antioxidan [54, 56].
26	Benzoic acid	-	0.22	0.55	C‡ H† O,	Phenolic compounds and food additives[57].
27	beta-Amyrin	-	1.19	-	$C_{30}H_{50}o$	Triterpenes, Anti-inflammatory[58].
28	1-Heptatriacotanol	-	0.64	-	$C_{37}H_{76}O$	Alcoholic compound, Antioxidant, Anticancer, Antimicrobial, Anti inflammatory[55, 59].
29	Phenol	-	-	6.22	C_6H_5OH	Phenolic compound and Antioxidant[60].

and sensitive to all *O. basilicum* extracts.

The results of MIC test showed that the widest MIC range was methanol extract from 3.125 to 25µg/mL then the range became progressively narrower with ethanol extract from 6.25 to 25µg/mL and water extract from 12.5 to 25µg/mL (Table. 2). *B. subtilis* was the most sensitive microbe to both alcoholic extracts, methanol extract with MIC 3.125µg/mL and ethanol extract with MIC 6.25µg/mL. All three *O. basilicum* extracts had inhibitory effect on both yeasts. *C. albicans* was more sensitive at MIC 12.5µg/mL than *C. tropicalis* with MIC 25µg/mL. MBC results showed that the ethanol extract have the highest value at 100µg/mL against *E. coli* also water extract against *E. coli* and *S. aureus.* The highest MFC value was 50µg/mL against *C. tropicalis* in all types of solvent compared to 25µg/mL against *C. albicans*. The result showed that the maximum ratio between MIC and MBC or MFC was three-fold (Table 2).

GC-MS analysis of *O. basilicum* extracts showed total components in ethanol extract, methanol extract and water extract were 18, 23 and 23 respectively (Table 3). The compounds higher than 4.0% were Propane, 3-chloro-1,1,1-trifluoro (19.84%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (19.26), n-Hexadecanoic acid (9.68%), 9,12,15-Octadecatrienoic acid, ethyl ester, (8.59%) and phytol (4.96%) in ethanol extract. In the methanol extract the compounds found were 4,5-Dichloro-1,3-dioxolan-2-one (24,23%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (10,47%), gamma –Sitosterol (11,52%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (6.30%), and stigmasterol (4.94%). And compounds found in water extract were 4,5-Dichloro-1,3-dioxolan-2-one (49.87%), phenol (6.22%), Propane, 3-chloro-1,1,1-trifluoro (4.26%) and gamma–Sitosterol (4.19%).

DISCUSSION

Lamiaceae family contains natural bioactive components that may be considered a promising field in the discovery of new antimicrobial agents. The present study showed that all tested bacteria and yeasts exhibit sensitivities towards *O. basilicum* extracts shown by the size of inhibition zone. The results of the statistical analysis showed significant differences between the three solvents (p-values < 0.01), except for the effect of the solvents on *C. albicans*, which did not have significant differences (p-value > 0.01. There are also significant differences among the microbes.

This result is in regard with a previous study[61] who found that water extract of *O. basilicum* inhibit the growth of both Gram positive and Gram negative bacteria. Moreover, Khalil[62] reported that ethanolic extract of *O. basilicum* inhibit the growth of *E. coil* and *S. aureus*. Also, our study partly agrees with another study found that aqueous extract of *O. basilicum* have stronger inhibitory activity comparing to ethyl acetate, methanol, and n-hexane extracts against Gram positive and Gram negative bacteria[24]. It is worth noting that the *O. basilicum* extracts showed inhibitory activity against *E. coil* and *S. aureus* that were resistance to the Erythromycin (E15 mcg), Indicating the superiority of the *O. basilicum* extracts on Erythromycin.

The present study showed that both yeasts were inhibited by ethanol, methanol and water extracts of *O. basilicum* this result are not consistent with a study conducted by Kaya *et al.*[63] where they found that acetone, chloroform and methanol extracts of *O. basilucum* did not show inhibitory activity against yeasts. These differences may be due to microbial strains, growth conditions, secondary metabolites, or extraction methods. The present result indicated that methanolic, ethanolic and water extracts of *O. basilucum* posses an antifungal activity.

The present results showed differences in inhibitory capability depending on the type of solvent in which the inhibitory activity for methanol extract was higher than ethanol and water against bacteria. This result is consistent with previous studies that confirm the effect of the solvent on the inhibitory capability of plant extracts and may return to solvent polarity[64], although the solvents used in this study are polar but vary in their strength in descending order water, methanol then ethanol[65]. In this study polar solvents have been chosen due to the ability of extracting many active compounds such as polyphenolic compounds[66].

The determination of MIC and MBC or MFC is important to measure the efficiency of the plant extract as antimicrobial agents[67]. The

MIC and MBC result showed that *O. basilicum* methanol extract had the lowest values of 3.125µg/mL and 6.25µg/mL against *B. subtilis*, respectively. In general, the present result showed that the MIC and MBC values of methanol extract are lower than those of the ethanol extract and the later are lower than the values of the water extract on the tested bacteria. This result supports the conclusion that antibacterial agents with low antibacterial activity have higher MIC and MBC values compared with more effective antibacterial agents[68]. However, the result showed that yeasts respond differently to inhibitory activity of *O. basilicum* extracts where the *C. albicans* was more sensitive than *C. tropicalis* in all the extracts. Where, the values of MIC and MFC required to inhibit the *C. albicans* are higher than the *C. tropicalis*.

Antimicrobial agents are considered as Microbicidal agents if the MBC or MFC is less than four times the MIC and Microbiostatic agent if the MBC or MFC is more or equal than four times the MIC[37]. Calculation of the ratio between MIC and (MBC or MFC) is shown to be less than three-fold. Accordingly inhibiting activity of *O. basilicum* extracts may be considered as Microbicidal agent.

The current result of GC-MS analysis of *O. basilicum* extracts showed the presence of several important chemical compounds like terpene, steroids, phenols, esters, and fatty acid. These entire compounds were reported to have a variety of biological activities (Table 3). This result is consistent with other studies reported that *O. basilicum* is rich in polyphenols like Phenolic acids[17,69]. It is interesting that the current study is not consistent with Murali and Prabakaran[27] whom found that *O. basilicum* methanol extract contains 13 compounds that did not resemble the chemical compounds found in this study. This may be due to the difference in the environment in which the plant is grown, the age of the plant or the extraction method.

The current study showed that the alcoholic and water extracts of O. *basilicum* were able to inhibit the growth of bacteria and yeast alike, and this may be due to the richness of O. *basilicum* with Phenolic compounds like Eugenol, Methyleugenol, Benzoic acid, Benzoic acid 4-hydroxy-, Salicylic acid, and Phenol. Phenolic compounds were reported to have several mechanisms of action against microbes, including changing the permeability of microbial cell membrane through accumulation of hydrophobic groups in the phospholipids bilayer disrupting the membrane integrity and leading to leakage of intracellular components and finally cell death. Also Phenolic compounds can bind to the enzymes inhibiting their functions like proteins, DNA and RNA synthesis[70,71]. Also the results showed that the O. *basilicum* extract contains terpene compounds like Phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Lupeol and beta-Amyrin that have an effect on microbial cell membranes by disrupting membrane efficiency.

Additionally, the antimicrobial activity may be related to the presence of fatty acids. The current result showed that O. *basilicum* extract were rich in saturated fatty acid and unsaturated fatty acid both are with long carbon chain 16 and more (Table 3). McGaw *et al.*[72] reported that Gram negative bacteria are less susceptible to fatty acids than Gram positive bacteria. Moreover, they reported that fatty acids carbon chain lengths play a very important role in their antimicrobial activity. Fatty acids containing 6 and less carbons inhibit Gram negative bacteria whereas Gram positive bacteria is inhibited by fatty acids with carbon chains longer than 12 and yeasts inhibited by fatty acids containing between 10 to 12 carbons. Several studies reported that unsaturated fatty acids with long carbon chains like linolenic acid, linoleic acid, and oleic acid have bactericidal effect on Methicillin-resistant *Staphylococcus aureus*, *Helicobacter pylori*, and *Mycobacteria*, while saturated fatty acids with long carbon chain like stearic acid and palmitic acid, are less active[73-75]. However, the primary molecular target of fatty acid is still unknown.

CONCLUSIONS

The current study showed that *O. basilicum* extracts have a broad inhibitory spectrum against Gram negative bacteria, Gram positive bacteria and yeast. The results showed that the *O. basilicum* extracts were able to inhibit *E. coil* and *S. aureus* that were resistant to Erythromycin (E15 mcg). Moreover, the results of the determination of the inhibitory efficiency showed that *O. basilicum* extracts possesses microbicidal properties. The results also showed

that the type of solvent is important in increasing the inhibitory capacity especially against the bacteria. The results of GC-MS showed that *O. basilicum* extracts are rich in compounds like Phenolic acids, fatty acid and terpene with inhibitory properties against wide range of microorganisms. The results of this study may provide new information on the importance of *O. basilicum* extracts in the production of broad-spectrum anti-microbial agents with micro-bicidal properties that are environmentally friendly, have no side effects compared to antibiotics and low cost.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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