

Antimicrobial Activities and Phytochemical Analysis of the Essential Oil of *Ocimum basilicum*, Collected from Jeddah Region, Saudi Arabia

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Abstract Multiple drug resistance bacteria have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. Thus, it should be searching for new antimicrobial agents from plants and detect its ability to treat diseases caused by resistant micro-organisms. *Ocimum basilicum* L. (Basil) is one of the most common plants used traditionally all over the world and in Saudi Arabia to treat many diseases. *O. basilicum* var. Genovese was collected from three different gardens in Jeddah city and the essential oils were extracted with either methanol or ethanol using Soxhlet. The results showed that the methanolic extract of *O. basilicum* was more active against pathogenic bacteria and fungi compared with the ethanol extract. Antibacterial activity was stronger against Gram positive than Gram negative bacteria. The highest activities were against *Bacillus* spp., *Micrococcus* spp. and *Staphylococcus aureus*. Moderate antifungal activity was recorded against fungi, *Fusarium* spp. was the most sensitive fungus. No activity was shown against all the tested pathogenic yeasts used in the study. The minimal inhibitory concentration (MIC) for bacteria and fungi were 25-75 µl/ml and 70-150 µl/ml respectively. The extracted oil proved to be effective on the wall composition of the most susceptible bacteria.

Keywords Antimicrobial activity, *Ocimum*, Essential oils, GC-MS, MIC

1. Introduction

Recently, multiple drug resistance in both human and plant pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [1, 2]. In addition to this trouble, antibiotics are sometimes associated with negative effects on the host, which includes hypersensitivity, immune-suppression, and allergic reactions [3]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources, including medicinal plants [4].

Many authors reported that, medicinal plants are a source of great economic value all over the world [5-10]. It is estimated that there are between 200,000 and 700,000 species of tropical flowering plants that have medicinal properties [11]. Their actions include antibacterial, antifungal, antiviral, anthelmintic, antiallergic, anticarcinogenic, analysis and larvicidal agents [12]. The beneficial medicinal effects of plant materials may typically

result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, essential oils, tannins, phenols, flavonoids, steroids, resins fatty acid gums, which are capable of producing definite physiological action on the microorganisms [13] and medically resistant strains of bacteria have been found to be widely inhibited by these compounds [14].

Many herb spices, especially those belonging to the Lamiaceae family, such as *Ocimum* showed strong antioxidant and antimicrobial activities [15]. Various species of *Ocimum* have been reported for their numerous medical uses [16]. *Ocimum* plant is widely spread in the world, which includes annual and perennial herbal plants, as well as shrubs, from tropical and subtropical zones of Asia, Africa and Central, and South America. The main center of diversity appears to be in Africa, further about 200 species of herbs and shrubs for this genus [17, 18].

The most important species of *Ocimum* genus are *O. sanctum* L. and *O. basilicum* L.; this latter species, usually named common basil or sweet basil and it is characterized by a considerable morphological and biochemical variability [19]. It will grow to a size of 1-2 feet in height [20]. Basil will prolifically produce large green leaves, measuring around 2 inches in length, throughout the summer [21]. Basil

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flowers are white, days to flowering are 100 days and are commonly removed to increase yield of leaves [18, 22]. It is very important for their therapeutic potential values. Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactagogue, stomachic and tonic agents [23-25]. The aims of this work were studied activity essential oils which extracted from aerial parts of *O. basilicum* var. Genovese against some pathogenic microorganisms as an antimicrobial.

Minimum inhibitory concentration (MIC) and the chemical composition of the basil oil in addition to its effect on the bacteria cell walls were also studied.

2. Materials and Methods

2.1. Tested Microorganisms

Tested microorganisms include the fungi *Penicillium glabrum*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* spp. and *Cladosporium* spp., the yeast *Candida albicans* and *Saccharomyces* spp. and the bacteria Methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus* spp. and *Micrococcus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Streptococcus pneumonia* and *Acinetobacter* spp. microorganisms were obtained from King Abdulaziz hospital, faculty of science, King Abdul Aziz University, and King Fahd Hospital, Jeddah, Saudi Arabia. The tested bacteria strains were maintained on the slopes of nutrient agar at 4°C [26]. All the organisms were regenerated every six months.

2.2. Tested Plant Extraction

Aerial parts including fresh leaves, flowers and wooden parts of *Ocimum basilicum* were collected from three different botanical gardens at north, central and south of Jeddah in Mars 2009, at 8 AM. The collected plant materials were put in clean plastic bags and transferred directly to the lab. The collected materials were cleaned and the infected or dead parts were excluded. The plant parts used were washed thoroughly using tap water and left to dry at room temperature for seven days. Then, the dried plant was pulverized using mechanical grinder. The powder was sieved with a 2 mm diameter mesh.

The plant was identified as *Ocimum basilicum* at Faculty of Science, Biology Department, King Abdul Aziz University. The complete identification was carried out at Botany Department, Faculty of Science, Tanta University Egypt, as *O. basilicum* var. Genovese.

By few modifications of [27] method: the dried and powdered plant materials (300 g) were extracted with 1 liter of either methanol or ethanol using Soxhlet (Electromantle ME) for 12 h at 90°C to obtain the essential oil of the plant.

Both methanolic and ethanolic extracts were concentrated by evaporating to dryness using rotary evaporator (Heidolph) at 40°C.

The essential oils obtained were collected and maintained according to [28] at 4°C until used in hermetically sealed dark glass bottles with rubber lids, covered with aluminium foil to protect the contents from light and kept under refrigeration at 4°C until used without any prior purification.

2.3. Antimicrobial Activities of *Ocimum basilicum* Extract

This test was carried out according to the method of [9]. 15 ml of sterilized nutrient agar (NA) for bacteria, potato dextrose agar (PDA) for fungi and Sabouraud agar for yeasts were poured into each Petri plate (90 mm diameter) and allowed to solidify. The plates were incubated with freshly prepared inoculums which were swabbed over the entire surface of the medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10^8 CFU/ml for bacteria, 10^4 spore/ml for fungi and 10^6 CFU/ml of yeast compared with 0.5 McFarland densities [29, 30]. One wheel of 6mm diameter was bored with the medium of each plate with the help of sterile cork-borer. 50 µl of essential oil was filled each well with the help of micropipette. Ampicillin (5µg/ml) was used as positive control against bacteria and amphotericin B (5µg/ml) was used as positive control against fungi and yeasts and sterile water as a negative control. Plates were left for 45 min at room temperature to allow proper diffusion of the extract to the medium. All plates were incubated at 37°C for 24 h, 28°C for 48 h and 37°C for 48 h for bacteria, fungi and yeasts respectively, and the inhibition zones were measured (mm). Inhibition of microbial growth was measured as the diameter of inhibition zone (mm) at 3-equidistant points taken from the center of the inhibition zone and the average value was calculated. All experiments were carried out in triplicate.

2.4. Determination of the Minimum Inhibitory Concentration (MIC)

This method was done as recommended by [31]. About 100 µl from each concentration of the plant extract are added to sterilize microtitre plate containing 100 µl of nutrient broth medium for bacteria and Sabouraud broth medium for fungi. Freshly prepared standard number of cells 10^6 CFU/ml for bacteria and 10^4 spore/ml for fungi were added plus some drops of phenol red. Growth or glucose metabolism was measured by a change of the color of phenol red indicator from red to yellow. Minimum inhibitory concentrations (MICs) are defined as no change in color of phenol red. For every experiment, sterile distilled water was used as negative control and standard antibiotics were included as a positive control. All experiments were carried out in triplicate.

2.5. Effect of *O. basilicum* Oil on Bacterial Cell Wall (mode of action)

The effect of the extracted oil was tested on *Bacillus* spp. The most sensitive bacteria in the study, cell wall was carried out on a morphological character using scanning electron microscope (SEM) and on the cell wall composition.

2.5.1. Isolation of Bacterial Cell Wall

The cell wall of *Bacillus* spp. was obtained using the method, which described by [32]. *Bacillus* spp. was cultivated in 250 ml Erlenmeyer flask containing 45 ml of nutrient broth, 2 ml bacterial suspension (10^8 CFU/ml) and 5 ml of the methanolic extract of *O. basilicum* or 5 ml sterile distilled water as negative control. Then, all flasks were incubated at 37°C for 24 h. After then, the bacterial cells were collected by centrifugation at 3000 rpm for 15 min to precipitate the cells. Cells were washed two times by sterile water and preservation at low temperatures. The bacterial cell walls were broken using cell Homogenizer (Ultra-Turrax 25) at low temperature for 5 min three times. After centrifugation at 3000 rpm for 30 min, the cell walls were collected and preserved at -20°C until used.

2.5.1.1. Quantitative Determination of Sugars, Protein and Phosphorous in Cell Wall

The determination of sugar was performed according to [33]. In the acidic medium, the concentrated sulfuric acid transformed the hexose or pentose sugars to furfural which can be condensed with the phenol to form a brown color complex. About 200 μ l of the digested cell walls were mixed with 10 μ l of 80% phenol in water and 1.5 ml conc. H_2SO_4 . The tubes were left for 5 min in a boiling water bath and then left at room temperature for 30 min. The absorbance was measured at A_{485} nm by using spectrophotometer (Perkin Elmer, USA) against a blank (200 μ l of the sterile distilled water was mixed with 10 μ l of 80% phenol in water and 1.5 ml conc. H_2SO_4). The quantity of the sugar can be determined from a standard curve of glucose.

The protein content of the cell wall was determined as described by [34]. The assay reagent was made by dissolving 100 mg of Coomassie Blue G250 in 50 ml of 95% ethanol. The solution was mixed with 100 ml of 85% phosphoric acid and made up to 1 L with distilled water. 1ml of the digested cell wall was mixed with 5 ml of assay reagent, mix well and the absorbance was measured at A_{595} nm. Blank was composed of 5 ml assay reagent plus 1ml sterile distilled water. The quantity of protein was determined from a standard curve of albumin.

Phosphor content was determinate spectrophotometrically. One ml of acid digested cell wall was neutralized by 8 N NaOH and one ml of ammonium molybdate sulfuric acid (25g NH_4MO_2 in 200 ml worm H_2O was filtrated above 400 ml of H_2O + 280 ml of conc. H_2SO_4 and completed to one

liter) followed by one ml of $SnCl_2 \cdot 2H_2O$ (0.5 gm $SnCl_2$ in 250 ml 2% HCl) were added. The contents were diluted to a certain volume, and the density of the color was determined using spectrophotometer at A_{700} [35]. The quantity was determined from a standard of KH_2PO_4 .

2.5.1.2. Imaging the Cells Using Scanning Electron Microscope (SEM)

Bacillus spp. was grown in 5ml nutrient broth with 2 ml methanolic oil as a treated sample and 2ml sterile distilled water as negative control. Then, all samples incubated at 37°C. After centrifugation at 3000 rpm for 30 min the cell walls were collected and prepared for using scanning electron microscopy (Quanta FEG 450) in unit of the electron microscope, King Abdulaziz University, Jeddah, Saudi Arabia.

2.6. Chemical analysis by Gas Chromatography–mass Spectrometry (GC/MS)

The essential oils of *O. basilicum* were rich with large number of compounds which considered as secondary metabolites. The chemical analysis was determined using gas chromatography -mass spectrometry (GC/MS) in faculty of Pharmacy, King Abdul Aziz University, Jeddah, Saudi Arabia.

2.7. Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS for windows, version 16) (SPSS Inc., Chicago, IL, U.S.A). The varying degree of the result is expressed as mean \pm standard deviation (Mean \pm SD). The significance of the difference between samples was determined using t-test. The difference was regarded significant when $P < 0.05$ and nonsignificant when $P > 0.05$, where P is a level of significance.

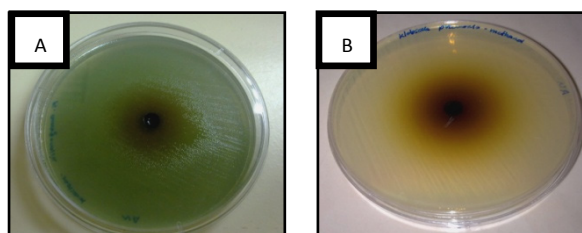
3. Results

3.1. Antimicrobial Activities of the Essential Oils

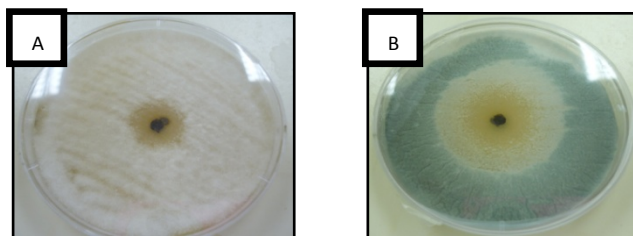
The results of the inhibit growth of the essential oil on the tested bacteria are shown in **Table 1** and **Figure 1**, the high inhibition was with the methanol extract and the most sensitive bacteria is *Bacillus* spp. with inhibition of 15.3 mm, then, *Micrococcus* spp., *Staphylococcus aureus* (MRSA), *S. aureus* and *E.coli* with inhibitions of 12, 12.7 and 12 respectively. In **Table 2** and **Figure 2** the results showed the inhibition of fungal growth after the treated with methanol or ethanol extracts, *Fusarium* spp. was the sensitive tested fungi with inhibition of 13 mm, *C. albicans* and *Saccharomyces* spp. were more resistant with no inhibition of growth appeared after the treated with both extracted.

Table 1. Antibacterial activity of essential oils extracted with methanol or ethanol using agar well diffusion method (inhibition diameters in mm) mean \pm SD

Tested bacteria	Gram reaction	Methanol* extract	Ethanol* extract	Positive control (Ampicilin)
<i>Bacillus</i> spp	+ ve	15.3 \pm 0.58	14 \pm 2	36
<i>Micrococcus</i> spp.	+ ve	13.3 \pm 1.5	13 \pm 1.2	38
<i>Staphylococcus aureus</i> (MRSA)	+ ve	12 \pm 1	8.7 \pm 1.2	17
<i>Staphylococcus aureus</i>	+ ve	12.7 \pm 0.58	9 \pm 1	38
<i>S. epidermidis</i>	+ ve	11 \pm 4	11.7 \pm 0.58	34
<i>Streptococcus pneumonia</i>	+ ve	10.7 \pm 0.58	NI	38
<i>Acinetobacte</i> spp.	- ve	10.7 \pm 2.5	9.3 \pm 2.3	20
<i>Escherishia coli</i>	- ve	12 \pm 2.6	10.3 \pm 1.5	35
<i>Klebsiella pneumonia</i>	- ve	11.3 \pm 0.58	NI	34
<i>Pseudomonas aeruginosa</i>	- ve	11 \pm 3.6	9 \pm 1.7	36
Antibacterial index		12	8.5	32.9
NI: no inhibition zone, + ve: Gram positive, - ve: Gram negative,				

**Figure 1.** Antibacterial activity of *Ocimum basilicum* essential oil on (A) *Pseudomonas aeruginosa* and (B) *Klebsiella pneumoniai***Table 2.** Antifungal and Antiyeasts activities of essential oils extracted with either methanol or ethanol using agar well diffusion method (inhibition zone diameters in mm) mean \pm SD

Fungi and yeast tested	Methanol* extract	Ethanol** extract	Positive control (Amphotericin B)
<i>Penicillium glabrum</i>	11.7 \pm 3	NI	22
<i>Aspergillus niger</i>	12.3 \pm 2.9	NI	19
<i>Aspergillus flavus</i>	9 \pm 2	NI	16
<i>Fusarium</i> spp.	13 \pm 1	12.7 \pm 4.9	15
<i>Cladosporium</i> spp.	NI	11.7 \pm 3	12
<i>Candida albicans</i>	NI	NI	23
<i>Saccharomyces</i> spp.	NI	NI	25
Antifungal Index	6.6	3.5	18.9
NI: No inhibition zone			

**Figure 2.** Antifungal activity of *Ocimum basilicum* essential oil on (A) *Aspergillus flavus* and (B) *Cladosporium* spp

3.2. Determination of the Minimum Inhibitory Concentration (MIC)

The results of *O. basilicum* MICs on tested microorganisms were showed in **Tables 3 and 4**, *Bacillus* spp., *Acinetobacte* spp, *E.coli* and *K. pneumonia* have the minimum inhibitory concentration with a value of 25 µl/ml *O. basilicum* methanol extracted. On other hand, *S. pneumonia*

and *K. pneumonia* have the highest minimum inhibitory concentration with the value more than 150 µl/ml *O. basilicum* ethanol extracted. *Fusarium* spp. recorded the less MICs on the tested fungi with values of 70, 75 µl/ml *O. basilicum* methanol and ethanol extracted respectively, while other tested fungi have the same value of MICs 150 µl/ml.

Table 3. MICs values (µl/ml) of *Ocimum basilicum* essential oils extracted with methanol or ethanol for the tested bacteria

Tested bacteria	Gram reaction	Methanol extract	Ethanol extract	Positive control (Ampicilin)
<i>Bacillus</i> spp.	+ ve	25±4.0	50±4.6	5±0.0
<i>Micrococcus</i> spp.	+ ve	50±6.1	75±4.5	5±1.0
(MRSA)	+ ve	50±2.0	75±4.0	5±0.0
<i>Staphylococcus aureus</i>	+ ve	50±3.3	75±4.0	5±2.0
<i>Staphylococcus epiderimdis</i>	+ ve	75±10.4	75±4. 5	5±0.0
<i>Streptococcus pneumonia</i>	+ ve	50±5.0	> 150±3.8	5±1.0
<i>Acinetobacte</i> spp.	- ve	25±5.9	50±3.2	25±3.0
<i>Escherishia coli</i>	- ve	25±1.9	50±5.2	25±4.0
<i>Klebsiella pneumonia</i>	- ve	25±4.0	> 150±4.0	25±2.0
<i>Pseudomonas aeruginosa</i>	- ve	50±4.0	50±4.0	15±1.0

+ ve: Gram positive, - ve: Gram negative

Table 4. MICs values (µl/ml) of *Ocimum basilicum* essential oils extracted with methanol or ethanol for the tested fungi

Tested fungi	MIC (µl/ml)		
	Methanol extract	Ethanol extract	Positive control (Amphotericin B)
<i>Penicillium glabrum</i>	150±4.0	150±8.0	15±1.0
<i>Aspergillus niger</i>	150±8.0	150±8.0	15±0.0
<i>Aspergillus flavus</i>	150±8.0	150±10.0	15±4.0
<i>Fusarium</i> spp.	70±10.0	75±8.0	15±40.0
<i>Cladosporium</i> spp.	150±10.0	150±10.0	15±0.0

Table 5. Quantity of sugar, protein and phosphorus in the cell walls of treated and untreated bacterial cells

mg/g	Cell walls	
	Normal cells	Treated cells
Sugars	300±21	116±14
protein	421±11	202± 22
phosphorus	97±11	111±17

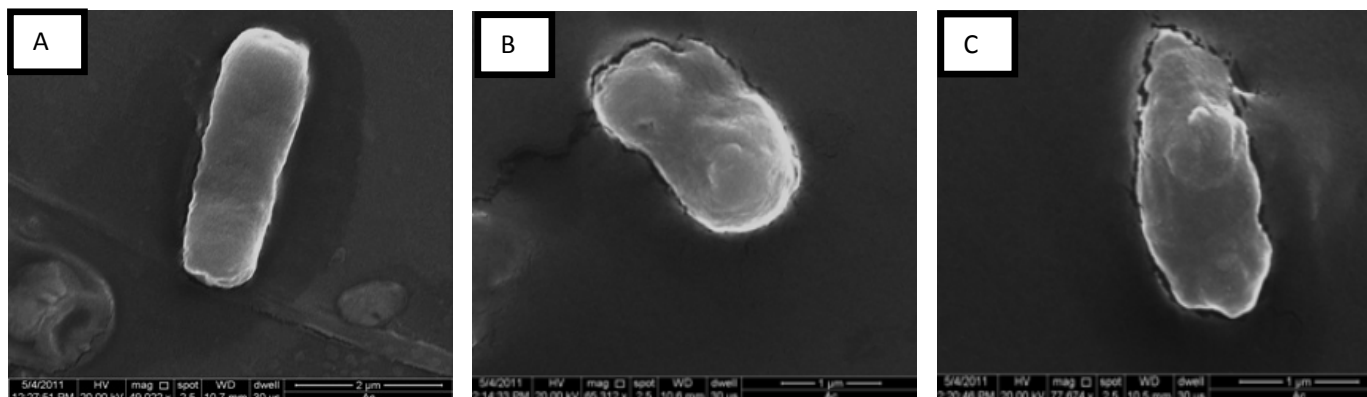


Figure 3. Scanning electron microscope of *Bacillus* spp. treated with *O. basilicum* methanol extract B and C compared with normal cell A.

3.3. Mode of Action: Effect of *O. basilicum* Oil on Bacterial Cell Wall

Table 5 detected the values of sugar, protein and phosphorus in *Bacillus* spp. cell wall, the results showed decreased of the sugar, protein content compared with the normal bacterial cell wall, and that means the *O. basilicum* methanol extract reduced the bacterial cell wall. Further, the content of phosphorus increased and that may due to the losses of energy components.

3.4. Imaging the Cells Using Scanning Electron Microscope (SEM)

Scanning electron microscope on **Figure 3** showed the damage effect of *O. basilicum* methanol extract on *Bacillus* spp. cell wall that appears on shrinkage, rupture, and partial deformation.

3.5. Chemical Analysis of Methanol and Ethanol Extract of *O. basilicum*

The results of chemical analysis of the methanol extract of *O. basilicum* were showed in **Figures 4 and 5**, the results detected presence of ten major components at different retention times. They were as follows: component was 1,6-Octadien-3-ol, 3,7-dimethyl-. Some of its synonymous names were β -Linalool and linalool, estragole and some of

its synonymous names were tarragon and estragole, linalool, trimethylsilyl ether, Trimethylsilyl ether glycerol and some of its synonymous names were Glycerol, tris (trimethylsilyl) ether and 2,2,8,8-Tetramethyl, tau.-Cadinol and some of its synonymous names: 4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol, trans- α -Bergamotene and some of its synonymous names: 2,6-Dimethyl-6-(4-methyl-3-pentenyl) bicycle [3.1.1] hept-2-ene, Benzene, 1,2-dimethoxy-4-(2-propenyl), tetradecanoic acid, trimethylsilyl ester, α -Linolenic acid, trimethylsilyl ester and 9, 12 Octadecadienoic (Z, Z)-, trimethylsilyl ester. While the effects of the chemical analysis of *O. basilicum* ethanolic extract showed the presence of five major components. The detected compounds are: 2-Furanmethanal, 5-ethenyltetrahydro- α , α , 5-trimethyl-, cis-, and some of its synonymous names were cis-Linalool Oxide. And linalool Oxide, 1,6-octadien-3-ol, 3,7-dimethyl-. Some of its synonymous names were β -Linalool and linalool, Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(1S)-, and some of its synonymous names were camphor and Levo(-)-camphor, 3,7-Octadiene-2,6-diol, 2,6-dimethyl-, some of its synonymous names were 1,5-Octadiene-3,7-diol, 3,7-dimethyl- and (3E)-2,6-Dimethyl-3,7-octadiene-2,6-diol, and Estragole, some of its synonymous names were tarragon and estragole.

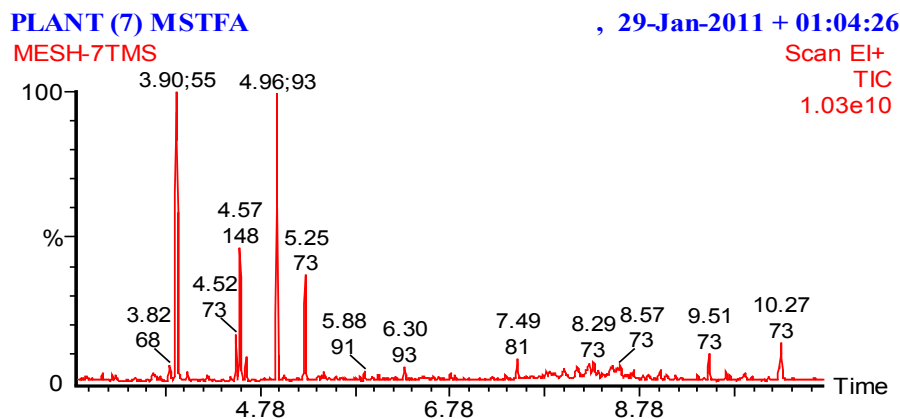


Figure 4. Chemical analysis of methanol extract of *O. basilicum* using GC/MS

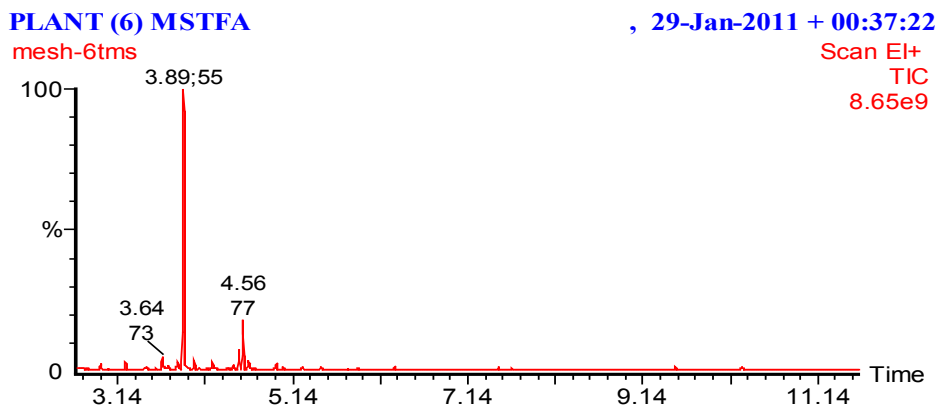


Figure 5. Chemical analysis of ethanol extract of *O. basilicum* using GC/MS

4. Discussion

The selection of *Ocimum basilicum* var. Genovese was due to its antimicrobial activities compared to other strains. In the same manner, **Carović-Stanko** [36] studied essential oils of the three botanical varieties and cultivars of *O. basilicum* ('Genovese', var. purpurascens and var. difforme) as an antibacterial agent. Their results indicated the greatest effectiveness was achieved by the essential oils from *O. basilicum* 'Genovese' which agrees with our study, but the essential oil of *O. basilicum* var. purpurascens and *O. basilicum* var. difforme showed the weakest antimicrobial activity.

The antimicrobial activities of the plants varied greatly with solvents because there are many factors that influence the active principles present in the plant which include the age of the plant, extracting solvent, method of extraction and time of harvesting plant materials [37, 38].

Based on the previously discussed results, the methanol extract has a stronger and broader spectrum of antimicrobial activities compared with the ethanol extracts, thus methanol was recommended for the extraction of the active antibacterial agents from *O. basilicum*. This result was in accordance with previous studies which reported that methanol was a better solvent for the more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water and ethanol [3, 4, 39].

In contrast, less antimicrobial activity was obtained by [40] who tested the activity of the ethanol extracts of ten plants, including *O. basilicum* plant, against 14 bacteria, the extracted oil was inactive against all the tested bacteria including *S. aureus*, *Ps. aeruginosa*, *K. pneumonia* and *E. coli*, but moderate activity was found only against one strain of *Ps. aeruginosa*. **Nedorostova et al.** [41] found excellent antimicrobial activity for *O. basilicum* extract against five foodborne bacteria. **Helal et al.** [42] noticed that the antimicrobial activity of all tested *O. basilicum* oils were generally higher against bacteria than fungi and yeast, which confirmed our results.

Bokhari [43] tested aqueous and organic extraction (ethyl alcohol, methanol, n-butanol, ethyl acetate or chloroform) of five medicinal plants growing in Jeddah including *O. basilicum* against species of some dermatophytes and all medicinal plants were active against the six tested dermatophytes. **Helal et al.** [42] tested the ability of essential oil extracted from five plants including *O. basilicum* against fungi, bacteria and yeasts in addition to bacteria associated with the contamination of fruit juices. The essential oils under test process showed excellent antimicrobial activity against fungi, bacteria and yeasts. They found that most of the *O. basilicum* oils inhibit conidia formation of the tested fungi, thus inhibiting fungal growth and flourish. Also, **Runyoro et al.** [44] found that essential oils of *Ocimum* species growing in Tanzania were active against three types of yeasts including *Candida albicans*, *C. tropicalis* and *C. glabrata*.

According to the survey that was conducted, there are very few studies about the antitumor activity of *O. basilicum* oils. Basil has shown antioxidant, antimicrobial and antitumor activities due to its phenolic acids and aromatic compounds [45, 46].

This work included a study of the mechanism the impact of essential oil of *O. basilicum* on the bacterial cell wall. Our results indicated that all Gram positive bacteria were more sensitive than Gram negative bacteria. Some studies agree with our findings. The antimicrobial activities *O. basilicum* against Gram-positive bacteria were higher than Gram-negative bacteria. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-negative has an outer membrane acting as a barrier to many environmental substances including antibiotics [47]. Gram-negative bacteria contain a high level of lipid materials. These materials are thought to make a substantial contribution to the mechanism whereby injurious chemicals are prevented from reaching their sites of action within the cell [48]. Perhaps to be expected, they possess an outer membrane surrounding the cell wall [49] which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering.

Gas chromatography-mass spectrometry apparatus was used to analyze and define the components of the essential oils which extracted from *O. basilicum* plant. This is the same method employed by [25, 50], who found that gas chromatography-mass spectrometry (GC-MS) has played the most important role in the identification of the chemical composition of basil essential oils.

The data recorded about finding in *O. basilicum* linalool and camphor is similar to the ones presented by the specific literatures, adding estragole. In a study carried by [51], it was found that inhaling linalool can reduce stress in lab rats. The findings could form the basis of new blood tests for identifying fragrances that can soothe stress. Many scientists have linked basil antimicrobial effects to the presence of high content of linalool.

5. Conclusions

Searching for new antimicrobial agents from plants and detect its ability to treat diseases caused by resistant microorganisms is needed. *Ocimum basilicum* L. (Basil) is one of the most common plants used traditionally all over the world and in Saudi Arabia to treat many diseases. Our results of the investigation of this plant showed its ability to be a new source of natural products used as antimicrobial agents.

REFERENCES

- [1] Davis, J. (1994) Inactivation of antibiotics and the dissemination of resistance genes, *Science*, vol.264: 375-382.

- [2] Service, R. F. (1995) Antibiotics that resist resistance, *Science*, vol. 270: 724- 727.
- [3] Ahmad, I.; Mehmood, Z. and Mohammad, F. (1998) Screening of some Indian medicinal plants for their antimicrobial properties, *Journal of Ethnopharmacology*, vol. 62: 183-193.
- [4] Cordell, G. A. (2000) Biodiversity and drug discovery(-) a symbiotic relationship, *Phytochemistry*, vol. 55: 463-480, 2000.
- [5] Kubo, L.; Muroi, H. and Himejima, M. (1993) Structure-antibacterial activity relationships of anacardic acids, *Journal Agricultural. Food Chemistry*, vol. 41: 1016-1019.
- [6] Shapoval, E. E. S.; Silveira, S. M.; Miranda, M. L.; Alice, C. B. and Henriques, A. T. (1994) Evaluation of some pharmacological activities of *Eugenia uniflora*, *Journal of Ethnopharmacology*, vol. 44: 136-142.
- [7] Artizzu, N.; Bonsignore, L.; Cottiglia, F. and Loy, G. (1995) Studies of the diuretic and antimicrobial activity of *Cynodon dactylon* essential oil, *Fitoterapia*, vol. 66: 174-175.
- [8] Ahmad, S. S. and Javed, S. (2007) Exploring the economic value of underutilized plant species in Ayubia National Park, *Pakistan Journal of Botany*, vol. 39(5): 1435-1442.
- [9] Joshi, B.; Lekhak, S. and Sharma, A. (2009) Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Organum majorana*, *Kathmandu University Journal Of Science, Engineering And Technology*, vol. 5 (I): 143- 150.
- [10] Joshi, B.; Sah, G. P.; Basnet, B. B.; Sharma, D.; Subedi, K.; Pandey, J. and Malla, R. (2011) Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem), *Journal of Microbiology and Antimicrobials*, vol. 3(1): 1-7.
- [11] Atata, R. F.; Sani, A. and Ajewole, S. M. (2003) Effect of stem bark extracts of *Enantia chloranta* on some clinical isolates, *Biokemistri*, vol. 15: 84-92.
- [12] Edeoga, H. O.; Okwu, D. E. and Mbuebie, B. O. (2005) Phytochemical constituents of some Nigerian medicinal plants, *African Journal of Biotechnology*, vol. 4: 685-688.
- [13] Joshi, A. R. and Edington, J. M. (1990) The use of medicinal plants by two village communities in the Central Development Region of Nepal, *Economic Botany*, vol. 44 (1): 71-83.
- [14] Akinyemi, K. O.; Oladapo, O.; Okwara, C. E.; Ibe, C. C. and Fasura, K. A. (2005) Screening of crude extracts of six medicinal plants used in South West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity, *BMC Complementary and Alternative Medicine*, vol. 5: 32-38.
- [15] Hirasa, K. and Takemasa, M. (1998) Spice science technology: New York: Marcel Dekker.
- [16] Mshana, N. R.; Abbiw, D/ K.; Addae-Mensah, I.; Adjanohoun, E.; Ahji, M. R. A.; Enow-Orock, E. G.; Gbile, Z. O.; Naomesi, B. K.; Odei, M. A.; Adenlami, H.; Oteng-Yeboah, A. A.; Sarppony, K.; Sofowora, A. and Tackie, A. N. (2000) Traditional medicine and pharmacopoeia contribution to the revision of Ethnobotanical and Floristic Studies in Ghana, Scientific, Technical and Research Commission of the Organisation of African Unity.
- [17] Paton, A. (1992) A synopsis of *Ocimum* L. (Labiatae) in Africa, *Kew Bul.*, vol. 47:403-435.
- [18] Simon, J. E.; Morales, M. R.; Phippen, W. B.; Vieira, R. F. and Hao, Z. (1999) Basil: a source of aroma compounds and a popular culinary and ornamental herb. In: Janick J (eds) Perspectives on new crops and new uses: Alexandria VA. ASHS Press, pp 499-505.
- [19] Telci, I.; Bayram, E.; Yilmaz, G.; and Avci, B. (2006) Variability in essential oil composition of Turkish basil (*Ocimum basilicum* L.), *Biochemical Systematic Ecology*, vol. 34: 489-497.
- [20] Boxer, A. and Back P. (1980) The Herb Book. London: Octopus Books, Limited.
- [21] Muenscher, W. C. and Rice M. A. (1978) Garden Spice and Wild Pot-Herbs. Ithaca, NY: Cornell University Press.
- [22] Duke, J. A. (1985) Ayensu ES. Medicinal Plants of China. Reference Publications. Inc.: ISBN 0-917256-20-4.
- [23] Lust J. (1983) The Herb Book. Bantam books: ISBN 0-553-23827-2.
- [24] Chiej R. (1984) Encyclopaedia of Medicinal Plants. MacDonald: ISBN 0- 356-10541-5.
- [25] Sajjadi, S. B. (2006) Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran, *DARU*, vol. 14 (3): 128-130.
- [26] Dadgar, T.; Asmar, M.; Saifi, A.; Mazandarani. M.; Bayat, H.; Moradi, A.; Bazueri, M. and Ghemi, E. (2006) Antibacterial activity of certain Iranian medicinal plants against Methicillin-Resistant and sensitive *Staphylococcus aureus*, *Asian Journal of Plant Sciences*, vol. 5 (5): 861-866.
- [27] Hakkim, F. L.; Arivazhagan G. and Boopathy R. (2008) Antioxidant property of selected *Ocimum* species and their secondary metabolite content, *Journal of Medicinal Plants Research*, vol. 2(9): 250-257.
- [28] Koba, K.; Poutouli, P.W.; Raynaud, C. and Sanda, K. (2009) Antifungal activity of the essential oils from *Ocimum gratissimum* L. grown in Togo, *Journal of Scientific Research*, vol. 1(1): 164-171.
- [29] Adigüzel, A.; Güllüce, M.; Şenqü, M.; Ögütçü, H.; Şahian, F. and Karaman, I. (2005) Antimicrobial effects of *Ocimum basilicum* (Labiatae) extract, *Turkish Journal Biotechnology*, vol. 29: 155-160.
- [30] Mihajilov-Krstev, T.; Radnović D. and Kitić D. (2010): Antimicrobial activity of *Satureja* L. essential oils against phytopathogenic bacteria *Erwinia amylovora*, *Biologica Nyssana*, vol. 1: 1-2.
- [31] Pijpers, E. A.; Noodergaaf, J.; Schoevers, E. and Verheijd, J. (1991) Comparison of methods for in vitro testing of susceptibility of Mycoplasma to antimicrobial agents., *Antimicrobial Agents and Chemotherapy*, vol. 35(2): 228-233.
- [32] Aly, M.M. (1997) Potency of certain actinomycetes for

certain antibiotic production. Ph.D. Cooperation between Tanta and Nancy Uni., Egypt -Frane, 350 pp.

- [33] Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances, *Analytical Chemistry*, vol. 28:350-356.
- [34] Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann, Biochemical Journal*, vol. 72: 248–254.
- [35] Allen, S. G.; Grimshaw, H. M.; Parkinson, J. A. and Quarmby, C. (1974) Chemical analysis of ecological materials. blackwell Sci. Puble. Oxford, london.565 pp.
- [36] Carović-Stanko, K.; Orlić, S.; Politeo, O.; Strikić, F.; Kolak, L.; Milos, M. and Satovic, Z. (2010) Composition and antibacterial activities of essential oils of seven *Ocimum* taxa, *Food Chemistry*, vol. 119: 196–201.
- [37] Okigbo, R. N. and Emoghene, I. A. (2003) Antifungal activity of leaf extracts of some plant species on *Mycosphaerella fijiensis* Morelet, the causal organism of black sigatoka diseases of banana (*Musa acuminata*), *King Mongkut's institute of Technology Ladkrabang Science Journal Thailand*, vol. 4: 20-31.
- [38] Okigbo, R. N. and Nmeka, I. A. (2005) Control of yam tuber rot with leaf extracts of *Xylopi aethiopica* and *Zingiber officinale*, *African Journal of Biotechnology*, vol. 4 (8): 804-807.
- [39] Elloff, J. N. (1998) Which extract should be used for the screening and isolation of antimicrobial components from plants?, *Journal Ethnopharmacology*, vol. 60: 1-8.
- [40] Nascimento, G. G. F.; Locatelli, J.; Freitas, P. C. and Silva, G.L. (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, *Brazilian Journal of Microbiology*, vol. 31: 247-256.
- [41] Nedorostova, L.; Kloucek, P.; Kokoska, L.; Stolcova, M. and Pulkrabek, J. (2009) Antimicrobial properties of selected essential oils in vapour phase against food borne bacteria, *Food Control*, vol. 20: 157–160.
- [42] Helal, G. A.; Sarhan, M. M.; Abu Shahla, A. N. K. and Abou El-Khair, E. K. (2006) Antimicrobial activity of some essential oils against microorganisms deteriorating fruit juices, *Mycobiology*, vol. 34(4): 219-229.
- [43] Bokhari, F. M. (2009) Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia, *Mycopath journal*, vol. 7(1): 51-57.
- [44] Runyoro, D.; Ngassapa, O.; Vagionas, K.; Aligiannis, N.; Graikou K. and Chinou, I. (2010) Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania, *Food Chemistry*, vol. 119: 311–316.
- [45] Gutierrez, B.; Ryan, C. and Bourke, P. (2008) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients, *International Journal of Food Microbiology*, vol. 124: 91-97.
- [46] Hussain, A. I.; Anwar, F.; Sherazi, S. T. H. and Przybylski, R. (2008) Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations, *Food Chemistry*, vol. 108: 986-995.
- [47] Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods—a review, *International Journal of Food Microbiology*, vol. 94: 223– 253.
- [48] Nwinyi, O. C.; Chinedu, N. S.; Ajani, O. O.; Ikpo, C. O. and Ogunniran, K. O. (2009) Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*, *African Journal of Food Science*, vol. 3(3): 077-081.
- [49] Ratledge, C. and Wilkinson, S. G. (1988) An overview of microbial lipids. In: Ratledge, C., Wilkinson, S.G. (Eds.), *Microbial Lipids*, vol. 1. Academic Press, London, pp. 3– 22.
- [50] Pripdeevech, P.; Chumpolsri, W.; Suttiarporn, P. and Wongpornchai, S. (2010) The chemical composition and antioxidant activities of basil from Thailand using retention indices and comprehensive two-dimensional gas chromatography, *Journal of the Serbian Chemical Society*, vol. 75 (11): 1503–1513.
- [51] Nakamura, A.; Fujiwara, S.; Matsumoto, I. and Abe, K. (2009) Stress Repression in Restrained Rats by (R)-(-)-Linalool Inhalation and Gene Expression Profiling of Their Whole Blood Cells, *Journal Agricultural. Food Chemistry, (Am. Chem. Soc.)*, vol.57 (12): 5480–5485.