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Full Length Research Paper

Antimicrobial activity of nonpolar natural product compounds of Egyptian *Ocimum basilicum* L

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In our study; light petroleum ether and diethyl ether extracts of Egyptian *Ocimum basilicum* L. were analyzed by Gas chromatography-mass spectrometer (GC-MS). Un-saponifiable part of the petroleum ether extract were found to contain twelve compounds with three main constituents were methyl eugenol (87.91%), 4,8,12-Trimethyl-1,3,7,11-tridecatetraene (5.11%) and phytol (3%), and the main constituents of saponifiable fraction of petroleum ether extract contained six fatty acid compounds with two main constituents were α –linoleic methyl ester (65.16 %) and linoleic methyl ester (18.18 %). Also un-saponifiable part of diethyl ether extract were found to contain eight compounds with two main constituents were Cyclopropane,1,1-dimethyl-2-(2,4-pentadienyl) (8.48 %) and 3-Methyl-3-buten-1-ol (5.54%), while saponifiable part gave a traces of free fatty acids. Antimicrobial activities were studied with five bacterial species (*Psudomonas aeruginosa*, *Kelbseilla* sp., *Salmonella typhi*, *Staphyllococcus aureus* and *E. coli*, and with five fungal species (*Fusarium oxysporum, Aspergillus flavus*, *Aspergillus niger*, *Cladosporium* sp. and *Penicillium* sp). Antibacterial activity showed that both extracts have resistance against all bacterial species at 3 mg concentration under investigation. While at 1 mg concentration both extracts have resistance against with *Psudomonas aeruginosa*, *Kelbseilla* sp. and *E. coli*. Also for antifungal activity we found both extracts have resistance against all species 3 mg concentration under investigation. While at 1 mg concentration of both extracts showed no resistance against all species.

Keywords: Antimicrobial activities, GC-MS, Hydrocarbons, Fatty acids and *Ocimum basilicum* L.

INTRODUCTION

Ocimum basilicum L. (basil) is an annual, herbaceous, white to purple flowering plant, 20–60 cm tall, that originated in Iran and India, (Chalchat and Özcan, 2008) (Özcan *et al.,* 2005). The taxonomy of *Ocimum* is complex due to interspecific hybridization and polyploidy of the species in the genus (Pushpangadan *et al.,* 1995), recognized more than 150 species; however, Paton (Paton *et al.,* 1999) proposed that *Ocimum* had only 65 species and other attributions should be considered as synonyms. Among the species of the genus, *Ocimum basilicum* L. (basil) is the major essential oil crop around the world and cultured commercially in many countries. Basil (*Ocimum basilicum* L.) is aromatic herb that is used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics, (Javanmardi *et al.,* 2002).

Generally, fatty acids of plant seed oils are classified as saturated and unsaturated fatty acids. Unsaturated fatty acids content, such as oleic, linoleic, includes arachidonic, eicosapentaenoic acids always higher than saturated fatty acids, except in coconut oil. In soybean oil, unsaturated fatty acid attained >90% (Bist *et al*., 2007), sown variety of *Safflower* oil >94% (Gurbuz *et al*., 2007). Among some unsaturated fatty acid, conjugated linolenic acid in tung seed oil, catalpa seed oil, pomegranate seed oil, bitter gourd oil, marigold seed oil and karela seed oil, are present in large quantities and

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can account for 31–80%, (Yang *et al*., *2005*) (Nagao and Yanagita, 2005) Meanwhile, the variety of basil seed oil contains of ALA range from 43.8–64.8% (Angers *et al*., *1996*).

The antibacterial activity of hexane, ethanol and aqueous extracts of each of 5 different medicinal plants namely; galangal, cabbage, eucalyptus, elecampane and basil were assayed against the growth of seven pathogenic bacteria representing two Gram-positive bacteria and five Gram-negative bacteria by disc diffusion method. The results revealed that basil hexane extract had broad-spectrum activity against all tested bacteria followed by galangal, eucalyptus, elecampane, and cabbage (Youssef *et al*., 2015).

MATERIALS AND METHODS

Collection of plant material

The plant was collected from Aswan city (south Egypt) in 2012, and dried in closed room in shadow then was identified by Botany Department, Faculty of Science, Zagazig University (Zagazig, Egypt).

Test micro-organisms

The bacterial and fungal strains were personally obtained from the microbiology Lab., Botany Department, Faculty of Science, Zagazig University. Bacterial species tested were *Psudomonas aeruginosa*, *Kelbseilla* sp., *Salmonella typhi*, *Staphyllococcus aureus* and *E. coli*; also fungal species were *Fusarium oxysporum, Aspergillus flavus*, *Aspergillus niger*, *Cladosporium* sp. and *Penicillium* sp.

Methods

Preparation of extracts

About 70 grams of the plant was extracted exhaustively with Pet. ether and Di ethyl ether using a Soxhlet apparatus. These were obtained the Pet. ether (60-80 ^{o}C) (3 g) and diethyl ether extracts (1.6 gm). The two extracts were collected and filtered through filter paper and concentrated on a Rota vapor at each temperature, then hydrolyses with alc. KOH (10%) and refluxing over water bath for 6 hours. Also dilution with water followed by extraction with ether afforded the ether part (un-saponifiable fraction) and (saponifiable fraction) potassium salt of fatty acid (Miura *et al*., 2003).

GC-MS (Gas Chromatography/Mass Spectrometry) analysis

The analytical GC-MS analyses were performed in two different equipment's: (a) Hewlett Packard 5973–6890 system, operating on EI mode and equipped with a HP 5 MS 30 m × 0.25 mm × 0.25 µ film thickness capillary columns. The carrier gas was Helium (flow rate = 1 mL/min). Temperature program: initial column temperature 60 °C (for 5 min.), was raised to 280°C within 3°C/min, and held there for 15 min. The injector and detector temperatures were 220 and 280 °C, respectively, (b) Finnegan trace GC ultra-system operating on EI mode and equipped with AT™ Aqua wax 30 m × 0, 32 mm × 0.25 µm film thickness capillary column. The carrier gas was Helium (flow rate = 1.5 ml /min, constant flow) and Split ratio, 1:10. Temperature program: initial column temperature 60 °C (for 5 min.), then was raised to 235 °C within 3 °C/min, and held there for 30 min (injector temperature 290 °C, detector temperature 300 °C). MS details (for both organs): ionization energy = 70 eV; emission = 200 µÅ; mass range = 35–650 Da; scan time = 1.25 s; scan rate (amu/s) = 500.0, scans/s = 0.7974.

All compounds were identified by comparison of their retention times ($\mathbf{R_t}$) and mass spectra with those of authentic samples and/or mainlib, Wiley 9, replib, NISTD-EMO libraries spectra and through international literature (Schumann and Siekmann, 2005).

Antimicrobial activities

Pretreatment of extract: Dissolving of P. ether and di ethyl ether extracts in dimethyl formamide (DMF) for antimicrobial investigation at the final concentration of (10mg / 1ml).

Antibacterial activity

Antibacterial activities of extracts were tested using pour plate technique on nutrient agar medium. Culturing and incubated of different bacterial species were carried out at 27 °C for 24 hours. Extracts were tested at two concentrations 0.1ml and 0.3ml (10mg / 1ml). After the elapse of incubation periods, the diameter of inhibition zones was measured (mm). Mean of 3 replicated was calculated. The inhibition zone formed by two extracts against the particular test bacterial strain determined as the antibacterial activities of the extracts (Vaghasiya *et al*., 2004).

Antifungal activity

Czapek Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup porer (15mm) diameter. Two concentrations 0.1ml and 0.3ml (10mg / 1ml) of the extracts were transferred into the well. Dimethyl formamide (DMF) was used only as a control. The plates were incubated for 7days at 30 °C. The inhibition zone (mm) formed by the extracts against the particular test fungal strain determined as the antifungal activities of the extracts.

Table 1. Shows the compounds of Hydrocarbons contents of the light –petroleum ether extract of *ocimum basilicum* L.

Rt	Chemical Composition	Area %	Molecular weight
17.06	2-Isononenal	0.09	140
17.75	Myrcenol	0.04	154
23.70	Methyl eugenol	87.91	178
24.50	Geraniol formate	0.86	182
26.52	Linalool oxide	0.04	170
28.10	10-Methyl-4-Undecene	0.04	168
29.6	3,3-dimethyl hexene	0.04	112
38.86	Phytol	3.00	296
45.28	2-Methyl hexadecane	0.05	240
50.21	4,8,12-Trimethyl-1,3,7,11-tridecatetraene	5.11	218
51.07	Tetradecane	0.29	198
53.72	2-Methyl heptadecane	0.47	254

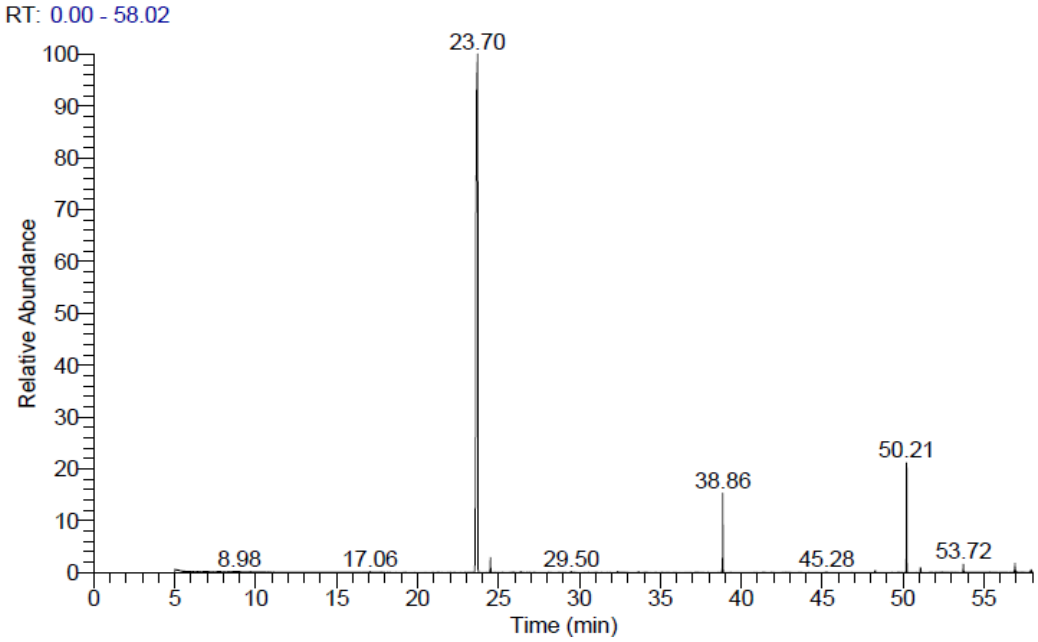

Figure 1. GC MS of Hydrocarbons contents of light petroleum extract of *ocimumbasilicum* L.

RESULTS AND DISCUSSION

After sequential extraction of the plant with two of the organic solvents are Pet. ether and Di ethyl ether by using soxhlet apparatus. We obtained two Pet. ether and diethyl ether extracts. For each extract hydrolyses with alc. KOH (10%) and diluted with water followed by ether afforded two fractions (saponifiable and un-saponifiable fractions). All fractions obtained were subjected to GC/MS to identify the chemical constituents of its one.

Hydrocarbons contents of the light-petroleum extract of Egyptian *Ocimum basilicum* L

The Un-saponifiable fraction was identified by GC/MS as shown in figure (1) and table (1), to contain twelve compounds:

2-Isononenal, Myrcenol, Methyl eugenol, Geraniol formate, Linalool oxide, 10-Methyl-4-Undecene, 3,3-dimethyl hexane, Phytol, 2-Methyl hexadecane, 4,8,12-Trimethyl-1,3,7,11-tridecatetraene, Tetradecane and 2-Methyl heptadecane.

From which the major compounds were methyl eugenol (87.91%), 4,8,12-Trimethyl-1,3,7,11-tridecatetraene (5.11%) and phytol (3%).

Table (**2**): show the compounds of the saponifiable part of the light –petroleum extract of *Ocimum basilicum* L.

Rt	Chemical composition	Area%	Molecular weight
6.10	Leuric methyl ester	1.21	214
10.14	Palmtic methyl ester	4.87	270
17.06	Oleic methyl ester	6.89	296
20.54	α –linoleic methyl ester	65.16	292
21.65	linoleic methyl ester	18.18	294
24.08	Stearic methyl ester	3.67	298

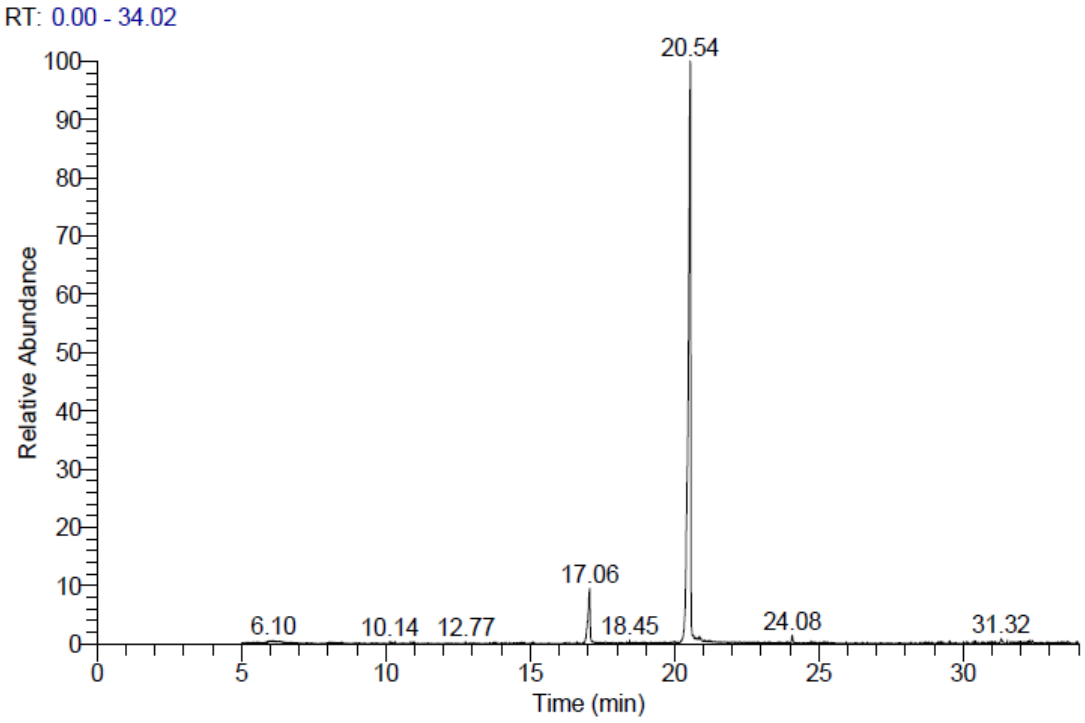

Figure 2. GC MS of The saponifiable part of the light petroleum extract of *Ocimum basilicum* L.

Fatty acids contents of light petroleum extract of Egyptian *Ocimum basilicum* L

The saponifiable fraction was acidified by dilute HCl till acidic medium then extracted with ether and methylated with diazomethane followed by analyzed GC/Ms. This extract was found as shown in figure (2) and table (2); to contain: six fatty acid compounds (Leuric methyl ester, Palmtic methyl ester, Oleic methyl ester, α –linoleic methyl ester, linoleic methyl ester and Stearic methyl ester), it was found that the main constituents were α –linoleic methyl ester (65.16 %) and linoleic methyl ester (18.18 %).

Hydrocarbons contents of Di ethyl ether extract Egyptian *Ocimum basilicum* L

Also the Un-saponifiable fraction was identified by GC/MS as shown in figure 3 and table (3), to contain eight compounds:

(1-Propyne, 2-Propanamine, Linalool oxide, 3-Methyl-3-buten-1-ol, 1-cyclohexyl ethanol, Cyclopropane,1,1-dimethyl-2-(2,4-pentadienyl), 5-Methyl -3-Hexen-2-one and Hexadecane), with two major compounds were Cyclopropane,1,1-dimethyl-2-(2,4-pentadienyl) (8.48 %) and 3-Methyl-3-buten-1-ol (5.54 %).

Table 3. show the compounds of the un-saponifiable part of the Di ethyl ether extract of *Ocimum basilicum* L.

Rt	Chemical Composition	Area %	Molecular weight
6.83	1-Propyne	0.76	40
9.67	2-Propanamine	2.85	59
12.91	Linalool oxide	0.97	170
14.9	3-Methyl-3-buten-1-ol	5.54	86
17.91	1-cyclohexyl ethanol	2.64	128
24.50	Cyclopropane,1,1-dimethyl-2-(2,4-pentadienyl)	8.48	136
25.76	5-Methyl -3-Hexen-2-one	1.11	112
38.88	Hexadecane	0.66	226

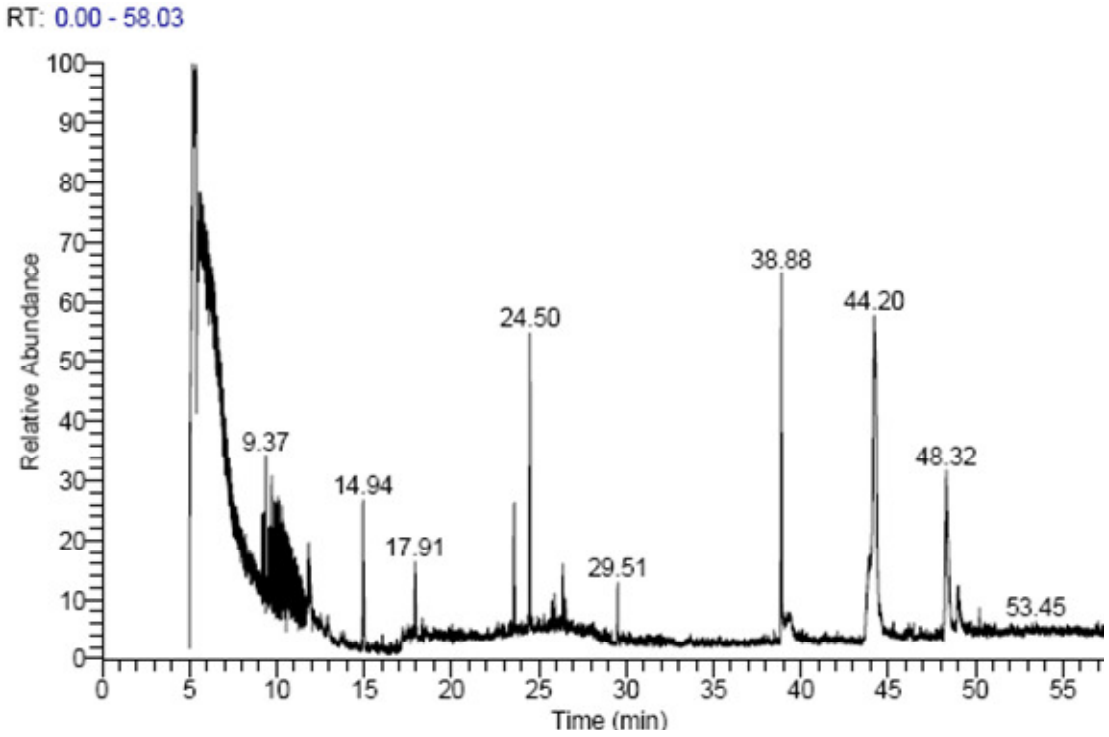

Figure 3. GC MS of The un-saponifiable part of the Di ethyl ether extract of *Ocimum basilicum* L.

Fatty acids contents of the Di ethyl ether extract of Egyptian *Ocimum basilicum* L

The saponifiable part (potassium salt of fatty acid) was then acidified with dil. HCL till acidic medium then extracted with ether , the extract give a traces of free fatty acids which was neglected.

Results of antimicrobial activity

Antimicrobial activities were studied with five bacterial species (*Psudomonas aeruginosa*, *Kelbseilla* sp., *Salmonella typhi*, *Staphyllococcus aureus* and *E. coli* and with five fungal species (*Fusarium oxysporum, Aspergillus flavus*, *Aspergillus niger*, *Cladosporium* sp. and *Penicillium* sp).

Results of antibacterial activity

Data found in figure (4); showed that both extracts have resistance against all bacterial species at 3 mg concentration under investigation. While at 1 mg concentration both extracts have resistance against with some species (*Psudomonas aeruginosa*, *Kelbseilla* sp. and *E. coli*).

High antibacterial activities due to mainly fatty acids especially unsaturated fatty acids in P. ether extract and activity with increase concentration of extract.

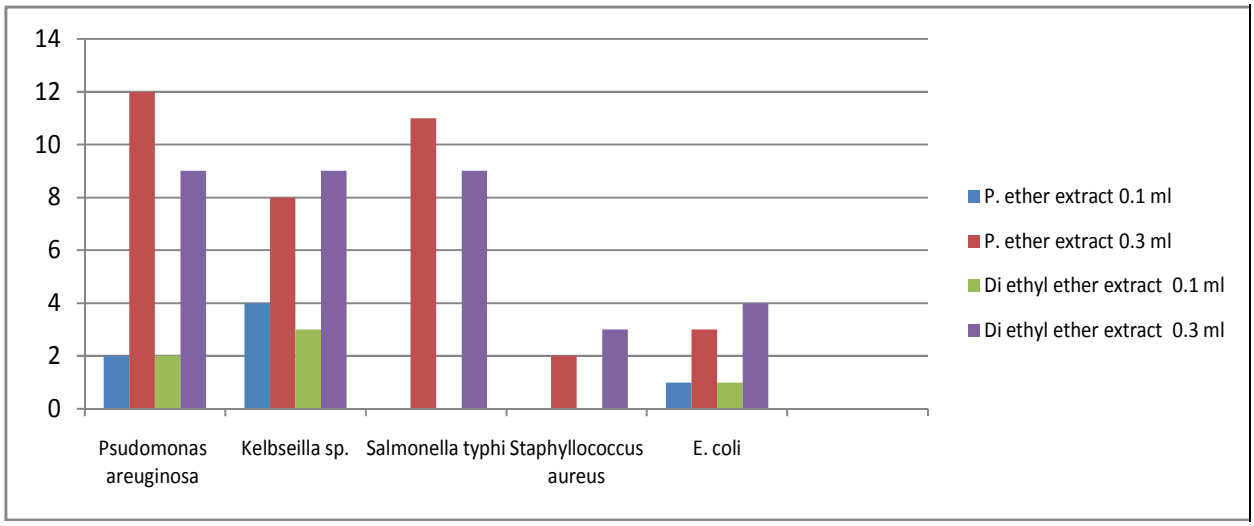

Figure 4. Antibacterial activity statically representation of P. ether and di ethyl ether extracts *Ocimum basilicum* L.

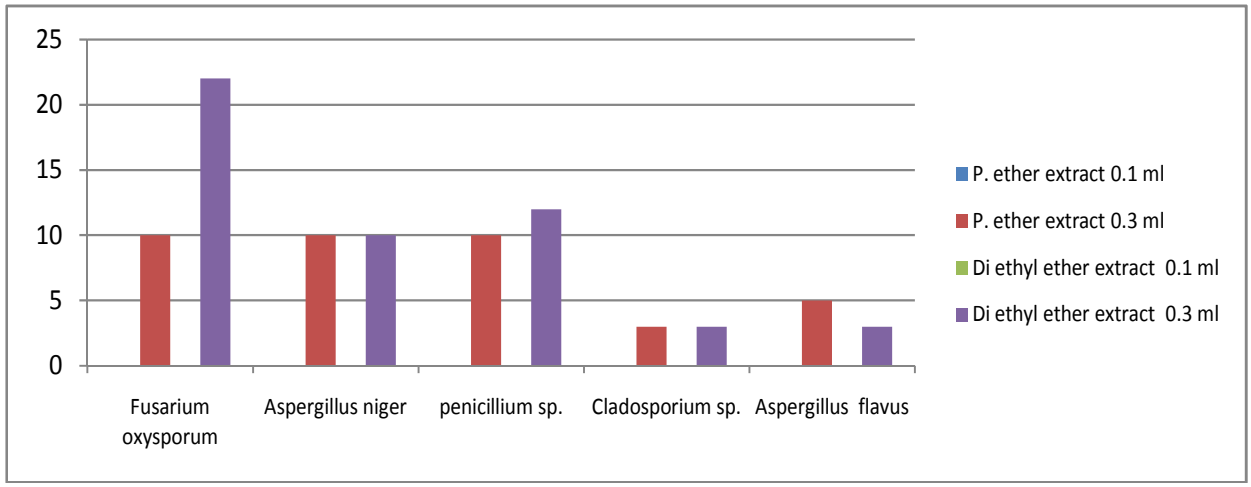

Figure 5. Antifungal activity statically representation of P. ether and di ethyl ether extracts *Ocimum basilicum* L.

Results of antifungal activity

Also for antifungal activity data as found in figure 5, showed that both extracts have resistance against all species 3 mg concentration under investigation. While at 1 mg concentration no resistance against all species.

CONCLUSION

We found that from the above results 12 hydrocarbon compounds and 6 fatty acids from P. ether extract while 8 hydrocarbon compounds from Di ethyl ether extract. Also the two extracts showed a promising antimicrobial activity at high concentration 0.3 mg.

REFERENCES

Angers P, Morales MR, Simon JR (1996). Fatty acid variation in seed oil among *Ocimum*s pecies. *J. Am. Oil Chem. Soc.* 73(3): 393–395

Bist S, Tao BY, Mohtar SA (2007). Method for Preparation, Use and Separation of fatty acid Esters. *United State Patent Application Publication*, Pub. No: US 2007/0251141 A1.

Chalchat JC, Özcan MM (2008). Comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) used as herb. *Food Chem.*, 110 (2): 501-503.

Gurbuz B, Cosge B, Kiralan B (2007). *Int. J. Nat. Eng. Sci.* 1(3):11–15.

Javanmardi J, Khalighi A, Kashi A, Bais HP, Vivanco JM (2002). Chemical characterization of basil (*Ocimum basilicum*L.) found in local accessions and used in traditional medicines in Iran, *J. Agric. Food Chem.* 50: 5878-5883.

Miura T, Ohta T, Miura CI, Yamauchi K (2003). Complementary deoxyribonucleic acid cloning of spermatogonial stem cell renewal factor *Endocrinol.* 144: 5504-5510.

Nagao K, Yanagita T (2005). *J. Biosci. Bioeng*.,100(2): 152–1578
Özcan M, Arslan D, Ünver A (2005). Effect of drying methods on the mineral content of basil (*Ocimum basilicum* L), *J. Food Eng.* 69 (3): 375-379.
Paton A, Harley RM, Harley MM (1999). Medicinal and Aromatic Plants-Industrial Profiles. Harwood Academic, Amsterdam, Pp. 1-38.
Pushpangadan P, Bradu BL (1995). Advances in Horticulture, Medicinal and Aromatic Plants, *vol. 11*. Malhotra Publishing House, New Delhi, Pp. 627-657.
Schumann K, Siekmann K (2005). Soaps" in Ullmann's Encyclopedia of Industrial Chemistry *Wiley-VCH, Weinheim*.
Vaghasiya YK, Nair R, Soni M, Balujs S, Chanda S (2004). Synthesis, structural determination and antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine. *J. Serbian Chem. Soc.* 69: 991.
Yang L, Leung KY, Cao Y, Huang Y, Ratnayake WMN, Zhen-Yu C (2005). α-Linolenic acid but not conjugated linolenic acid is hypocholesterolaemic in hamsters, *Br. J. Nutr.* 93: 433–438.
Youssef MS, Saber SM, Hassane AMA, Arafa RF (2015). Evaluation of Antibacterial Activities of Some Egyptian Medicinal Plant Extracts. *J. Ecol. Health Environ.* 3: 49-57.