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Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatographymass spectrometry (GC-MS) techniques

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The objective of this research was to determine the chemical composition of aerial parts extract from methanol. The phytochemical compound screened by gas chromatography-mass spectrometry (GC-MS) method. Thirty one bioactive phytochemical compounds were identified in the methanolic extract of *Ocimum basilicum*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, MS fragment-ions and pharmacological actions. GC-MS and Fourier transform infrared (FT-IR) analyses of *O. basilicum* revealed the existence of Paromomycin, Stevioside, Campesterol and Ascaridole epoxide, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, alkanes, H-bonded H-X group, hydrogen bonded alcohols and phenols. Methanolic extract of bioactive compounds of *O. basilicum* was assayed for *in vitro* antibacterial activity against *Pseudomonas aerogenosa, Proteus mirabilis, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* by using the diffusion method in agar. The zone of inhibition was compared with different standard antibiotics. The diameters of inhibition zones ranged from 5.70±0.10 to 0.55±0.29 mm for all treatments.

Key words: GC/MS, Bioactive compounds, Fourier transform infrared (FT-IR), Ocimum basilicum.

INTRODUCTION

Plants secondary metabolites have recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities (Rukayadi et al., 2006; Yoshikawa et al., 2007; Hameed et al., 2015a; Al-Marzoqi et al., 2016). It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> effect (Halliwell and Gutteridge, 1992; Altameme et al., 2015a). Plant materials have played an important role in traditional methods of field crop and stored grain protection against insect pest infestation since time immemorial (Ogendo et al., 2003; Al-Marzoqi et al., 2015). Ocimum species contains a wide range of essential oils rich in phenolic compounds and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins. The genus Ocimum comprises more than 150 species and is considered as one of the largest genera of the Lamiaceae family (Holm, 1996; Hameed et al., 2015b). Ocimum basilicum L. (sweet basil) is an annual herb which grows in several regions all over the world. The plant is widely used in food and oral care products. The essential oil of the plant is also used as perfumery (Bauer et al., 1997; Chiang, 2005). Kéita et al. (2000) reported O. basilicum and O. gratissimum to be potential insecticides. The leaves and seeds are rich in essential oils, which are repellent, toxic or growth inhibitory to many insects. A high degree of polymorphism in the aenus Ocimum determines a large number of subspecies, different varieties and forms producing essential oils with varying chemical composition offering variable level of medicinal potential. Essential oils extracted from Ocimum plants have been reported to possess interesting biological properties. These volatile oils have been applied in perfumery, to inhibit growth of food preservation microorganisms, in and in aromatherapy. The potential uses of O. basilicum, Ocimum canum Ocimum gratissimum and Ocimum sanctum essential oils, particularly as antioxidant and antimicrobial agents have also been explored (Politeo et al., 2007; Koba et al., 2009; Zhang et al., 2009; Hameed et al., 2015c; Altameme et al., 2015b; Hussein et al., 2016a). O. basilicum has been traditionally used for the treatment of many ailments, such as headaches, coughs and diarrhea and it is generally recognized as safe and is a rich source of phenolic antioxidant compounds and flavonoids (Juliani and Simon, 2002). The aims of this study were to determine the phytochemical composition of aerial parts and evaluation of anti-bacterial activity.

MATERIALS AND METHODS

Collection and preparation of plant

The aerial parts were dried at room temperature for ten days and when properly dried then powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve (Altameme et al., 2015c; Hameed et al., 2015d; Hussein et al., 2016b). The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature. About 9 g of the plant sample powdered were soaked in 100 ml methanol individually. It was left for 72 h so that alkaloids, flavonoids and other constituents if present will get dissolved (Jasim et al., 2015; Hadi et al., 2016; Hussein et al., 2016c). The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed.

Gas chromatography-mass spectrum (GC-MS) analysis

The GC-MS analysis of the plant extract (O. basilicum) was made in a Agilent 7890 A instrument under computer control at 70 eV. About 1 µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the retention time (RT). The M/Z (mass/charge) ratio obtained was calibrated from the graph obtained, which was called as the mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1 ml/min. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries (Yang et al., 2010).

Determination of antibacterial activity of crude bioactive compounds of *O. basilicum*

The test pathogens (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus*) were swabbed in Muller Hinton agar plates. $60 \ \mu$ l of plant extract was loaded on the bored wells. The wells were bored in 0.5 cm in diameter. The plates were incubated at 37C° for 24 h and examined. After the incubation the diameter of inhibition zones around the discs was measured.

RESULTS AND DISCUSSION

Medicinal herbs are known as sources of active compounds that are widely sought after worldwide for their natural properties. They have been used since ancient times as sources of flavorings and for their pharmaceutical properties (Bais et al., 2002). Phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992). A great number of organizations and scientists turn their attention to traditional therapies in order to find and conserve important resources and up to 80% of the population relies on traditional medicines or folk remedies for primary health care needs (Smith et al., 2010). Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic aerial parts extract of O. basilicum, shown in Table 1. The GC-MS chromatogram of the 31 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of O. basilicum showed the presence of thirty one major peaks and the components corresponding to the peaks were determined

RT Phytochemical Molecular **MS Fragment- ions** Pharmacological actions S/N Chemical structure Formula Exact mass weight compound (min) HO. ŝ 1,2,4-Triazole , 4-[N-(2-Biological activities including 3.196 173.054889 1 hydroxyethyl)-N- $C_4H_7N_5O_3$ 173 55,70,83,113,127,173 anti- microbial activity nitro]amino Antimicrobial and anti -2 9-Acetoxynonanal 3.459 C11H20O3 200 200.141245 55,81,97,112,157,200 inflammatory 57,67,80,94,109,124,162,191,21 3 Paromomycin 3.567 C23H45N5O14 615 615.296303 Anti-bacterial H2N-0,227,244,262,287 OH Have anti-hyperglycemic, 60,73,85,98,113,121,144,163,18 Stevioside 3.773 C38H60O18 804 804.377964 4 anti-hypertensive anti-5,214,260,285 inflammatory and anti-tumor 5 136 136.1252 D-Limonene 3.985 C10H16 53,68,79,93,136 Anti-stress effects

Table 1. Phytochemical compounds identified in methanolic extract of O. basilicum.







22	Lup-20(29)-en-28-oic acid , 3-hydroxy-, methyl ester , (3ß)-	17.317	C31H50O3	470	470.375996	107,175,189,207,220,262,341,4 11,452,470	<i>Anti</i> -bacterial
23	Octadecanoic acid	17.186	C ₁₈ H ₃₆ O ₂	284	284.27153	60,73,83,97,115,129,143,157,17 1,185,199,227,241,255,284	Antiviral and <i>anti-</i> inflammatory activities
24	2-[4-methyl-6-(2,6,6- trimethylcyclohex-1- enyl)hexa-1,3,5- trienyl]cyclol	17.369	C23H32O	324	324.245316	55,69,79,91,105,135,173,187,23 9,269,324	Unknown
25	9-Desoxo-9-x-acetoxy- 3,8,12-tri-acetylingol	21.946	C ₂₈ H ₄₀ O ₁₀	536	536.262146	55,69,122,207,236,297,357,417, 477	Anti-macrofouling
26	9,12-Cyclolanost-24-en- 3-ol, acetate,(3ß)-	23.497	$C_{32}H_{52}O_2$	468	468.39673	55,69,81,95,175,203,231,286,40 8,468	<i>Anti</i> -inflammatory





Figure 1. GC-MS chromatogram of methanolic extract of Ocimum basilicum.



Figure 2. Structure of 1,2,4-Triazole, 4-[N-(2-hydroxyethyl)-N-nitro]amino with 3.196 (RT) present in *Ocimum basilicum*.

as follows. The first set up peak were determined to be 1,2,4-Triazole (Figure 2). The second peak indicated to



Figure 3. Structure of 9-Acetoxynonanal with 3.459 (RT) present in *Ocimum basilicum*.

be 4-[N-(2-hydroxyethyl)-N-nitro]amino (Figure 3). The next peaks were considered to be, 9-Acetoxynonanal,



Figure 4. Structure of Paromomycin with 3.567 (RT) present in *Ocimum basilicum*.



Figure 5. Structure of Stevioside with 3.773 (RT) present in *Ocimum basilicum*.

Paromomycin, Stevioside, D-Limonene, Exo-2,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol, Dithiocarbamate, Smethyl-N-(2-methyl-3-oxobutyl)-, 1,6-Octadien-3-ol,3,7dimethyl-, 13,16-Octadecadiynoic acid , methyl ester, Cyclohexanecarboxylic acid ,2-hydroxy-, ethyl ester, Methyl 6-oxoheptanoate, 3,7-Octadiene-2,6-diol,2,6dimethyl-, Exo-2,7,7-trimethylbicyclo [2.2.1] heptan-2-ol,



Figure 6. Structure of D-Limonene with 3.985 (RT) present in *Ocimum basilicum*.



Glycyl-D-asparagine, 6-Acetyl-ß-d-mannose, 2-Propenoic acid, 3-phenyl-, methyl ester, (E)-, Methyleugenol, 7-epicis-sesquisabinene hydrate, Mannopyranose, 1-0-(triethylsilyl)-, 2,3:4,6-dibutaneboronate, 2,6-Bis[2-[2-Sthiosulfuroethylamino] ethoxy]pyrazine, 9,12,15-Octadecatrienoic acid , (Z,Z,Z)-, Lup-20(29)-en-28-oic acid, 3-hydroxy-, methyl ester, (3ß)-, Octadecanoic acid, 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5trienyl]cyclol, 9-Desoxo-9-x-acetoxy-3,8,12-tri-acetylingol, 9,12-Cyclolanost-24-en-3-ol, acetate,(3ß)-, y-Tocopherol, Lup-20(29)-en-3-ol,acetate,(3ß)-, Ethyl iso - allocholate, Campesterol and Ascaridole epoxide (Figures 4 to 32).



Figure 8. Structure of Dithiocarbamate , S-methyl-N-(2-methyl-3-oxobutyl) with 4.775 (RT) present in *Ocimum basilicum*.



Figure 9. Structure of 1,6-Octadien-3-ol,3,7-dimethyl with 5.067 (RT) present in *Ocimum basilicum*.



Figure 10. Structure of 13,16-Octadecadiynoic acid , methyl ester with 5.301 (RT) present in *Ocimum basilicum*.



Figure 11. Structure of Cyclohexanecarboxylic acid ,2-hydroxy-, ethyl ester with 5.776 (RT) present in *Ocimum basilicum*.



Figure 12. Structure of Methyl 6-oxoheptanoate with 6.011 (RT) present in *Ocimum basilicum*.



Figure 14. Structure of Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol with 6.303 (RT) present in *Ocimum basilicum*.



Figure 13. Structure of 3,7-Octadiene-2,6-diol,2,6-dimethyl with 6.245 (RT) present in *Ocimum basilicum*.



Figure 15. Structure of Glycyl-D-asparagine with 6.772 (RT) present in *Ocimum basilicum*.



Figure 16. Structure of 6-Acetyl-ß-d-mannose with 6.932 (RT) present in *Ocimum basilicum*.



Figure 17. Structure of 2-Propenoic acid ,3-phenyl-,methyl ester , (E) with 7.744 (RT) present in $\it Ocimum \ basilicum.$



Figure 18. Structure of Methyleugenol with 8.980 (RT) present in *Ocimum basilicum*.



Figure 19. Structure of 7-epi-cis-sesquisabinene hydrate with 9.404 (RT) present in *Ocimum basilicum*.



Figure 20. Structure of Mannopyranose , 1-O-(triethylsilyl)-, 2,3:4,6dibutaneboronate with 13.163 (RT) present in *Ocimum basilicum*.



Figure21.Structureof2,6-Bis[2-[2-S-thiosulfuroethylamino]ethoxy]pyrazinewith16.133 (RT) present inOcimum basilicum.



Figure 22. Structure of 9,12,15-Octadecatrienoic acid , (Z,Z,Z) with 17.031 (RT) present in *Ocimum basilicum*.



Figure 23. Structure of Lup-20(29)-en-28-oic acid , 3-hydroxy-, methyl ester , (3ß) with 17.317 (RT) present in *Ocimum basilicum*.



Figure 24. Structure of Octadecanoic acid with 17.186 (RT) present in *Ocimum basilicum*.



Figure 26. Structure of 9-Desoxo-9-x-acetoxy-3,8,12-tri-acetylingol with 21.946 (RT) present in *Ocimum basilicum*.



Figure 25. Structure of 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1enyl)hexa-1,3,5-trienyl]cyclol with 17.369 (RT) present in *Ocimum basilicum*.



Figure 27. Structure of 9,12-Cyclolanost-24-en-3-ol, acetate,(3ß) with 23.497 (RT) present in *Ocimum basilicum*.



Figure 28. Structure of y-Tocopherol with 25.231 (RT) present in *Ocimum basilicum.*



Figure 30. Structure of Ethyl iso – allocholate with 26.890 (RT) present in *Ocimum basilicum*.



Figure 29. Structure of Lup-20(29)-en-3-ol,acetate,(3ß) with 26.467 (RT) present in *Ocimum basilicum*.



Figure 31. Structure of Campesterol with 28.229 (RT) present in *Ocimum basilicum*.



Figure 32. Structure of Ascaridole epoxide with 7.167 (RT) present in *Ocimum basilicum*.

Table 2. FT-IR	peak values of	Ocimum	basilicum metha	nolic aerial	parts extract.

No.	Peak (Wave number cm-')	Intensity	Bond	Functional group assignment	Group frequency
1	665.44	61.240	-	Unknown	-
2	1028.06	60.648	C-F stretch	Aliphatic fluoro compounds	1000-1050
3	1095.57	64.096	C-0	Alcohols, ethers, carboxlic acids, esters	1050-1300
4	1139.93	67.976	C-0	Alcohols, ethers, carboxlic acids, esters	1050-1300
5	1155.36	67.976	C-0	Alcohols, ethers, carboxlic acids, esters	1050-1300
6	1238.30	72.739	C-0	Alcohols, ethers, carboxlic acids, esters	1050-1300
7	1317.38	76.675	NO ₂	Nitro compounds	1300-1370
8	1361.74	75.877	NO ₂	Nitro compounds	1300-1370
9	1396.46	75.235	C-H	Alkanes	1340-1470
10	1616.35	74.205	-	Unknown	-
11	1734.65	72.915	-	Unknown	-
12	2852.72	79.425	C-H	Alkanes	2850-2970
13	2924.09	73.457	C-H	Alkanes	2850-2970
14	3010.88	84.980	H-O	H-bonded H-X group	2500-3500
15	3196.05	83.617	H-O	H-bonded H-X group	2500-3500
16	3275.13	81.882	O-H	Hydrogen bonded alcohols, phenols	3200-3600

The FTIR analysis of *O. basilicum* aerial parts proved the presence of aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, alkanes, H-bonded H-X group, hydrogen bonded alcohols and phenols which shows major peaks at 1028.06, 1095.57, 1238.30, 1317.38, 1396.46, 1396.46, 1616.35, 3010.88 and 3275.13 (Table 2 and Figure 33). Baritaux et al. (1992) isolated and analyzed essential oil from basil (*O.* *basilicum*) by steam distillation, then it were recovered into a pentane/dichlorometane mixture containing known amounts of two internal standards, nonane and octadecane. Laakso et al. (1990) analyzed essential oil from holy basil by two extraction methods steam distillation and water distillation. The essential oil was identified into 20 different compounds by GC-MS and GCFTIR. They contained high amounts of eugenol



Figure 33. FT-IR peak values of Ocimum basilicum.

Table 3. Zone of inhibition (mm) of test bacterial strains to Ocimum basilicum bioactive compounds and standard antibiotics.

	Bacteria							
Ocimum basilicum antibiotics	Staphylococcus	Escherichia	Proteus	Klebsiella	Pseudomonas			
	aureus	coli	mirabilis	pneumonia	eurogenosa			
Ocimum basilicum	5.00±0.21	5.70±0.10	4.30±0.20	4.22±0.23	4.17±0.44			
Rifambin	0.89±0.22	0.86±0.31	1.00±0.35	0.96±0.26	2.00±0.06			
Streptomycin	1.09±0.33	1.44±0.29	0.99±0.28	1.00±0.47	1.64±0.30			
Kanamycin	0.55±0.29	0.98±0.28	2.01±0.12	0.69±0.23	1.50±0.18			
Cefotoxime	1.57±0.27	1.86±0.32	1.03±0.24	1.00±0.23	0.95±0.27			

(24.2%), β-bisabolenes (15.4%), α-bisabolenes (10.6%) and methyl chavicol (11.16%), but methyleugenol could not be detected. Raju *et al.* (1999) analyzed bioactive chemical compounds isolated in the leaves of basil from India by GC and GC-MS. Twenty-five components were found from 98.7% total oil area. Eugenol (53.4%), βcaryophyllene (31.7%) and β-elemene were found as the major components. Essential oils are extracted from various aromatic plants generally located in temperate to warm countries, like Brazil, where they represent an important part of the traditional pharmacopoeia due to their important biological activities (Morales and Simon, 1996; Phippen and Simon, 1998). An important approach to discover new medicines is survey of natural products, such as medicinal plants or their secondary metabolites that modulate painful conditions (Baratta et al., 1998; Chiang et al., 2005).

Previous studies (Bakkali et al., 2008; Quintans-Júnior et al., 2008; Oliveira et al., 2009; Li and Vederas, 2009; Venâncio et al., 2011) have demonstrated that several *Ocimum* species are used to treat central nervous system (CNS) disorders in various regions of the world, mainly in developing countries, and their analgesic profile is frequently reported. In this study, five clinical pathogens were selected for antibacterial activity, namely, *S. aureus, K. pneumoniae, P. aeruginosa, E. coli* and *Proteus mirabilis.* Maximum zone formation is against *E. coli* (5.70±0.10) (Table 3). Essential oils derived from several

Ocimum spp. have been reported to be active against several Gram-positive and Gram-negative bacteria as well as against yeasts and fungi due to their terpenic constituents. Recently, essential oils and extracts of certain plants have been shown to have antimicrobial effects as well as imparting flavour to foods (Zhang et al., 2009; Shareef et al., 2016; Kadhim et al., 2016). Ocimum spp. contain a wide range of essential oils rich in phenolic compounds and a wide array of other natural products polyphenols such as flavonoids includina and anthocyanins. Antiviral and antimicrobial activities of this plant have also been reported (Li and Vederas, 2009; Venâncio et al., 2011).

Conclusion

The results of this study provide data on phytochemical characteristics of O. basilicum. O. basilicum is native plant of Iraq. It contains chemical constitutions which may be useful for various herbal formulation as antiinflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.

Conflict of interest

The authors have not declared any conflict of interest

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