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Volatile Oil Composition of *Ocimum basilicum* (Rehan) Leaf Extract and Antibacterial Activity Against Bacterial Pathogens in Sudan

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ABSTRACT

The dried leaves of *Ocimum basilicum* 100 g, produced 8.25% of concentrated volatile oil by hydro distillation method, the constituent of the oil were examined by GC-MS, it contained, Methyl eugenol (54.02%), Linalool (21.34%)α-cubebene (5.200%), Limonene (1.01%) nerol (0.875%), epsilon-muurolene(0.831%)α-pinene (0.76%) as the major compounds. The effect of volatile oil of *Ocimum basilicum* L, against six different pathogenic bacteria *Staphylococcus aureus, Escherichia coli, Pseudomona aeruginosa, Salmonella typhimurium, Klebsiella pneumonia and Bacillus cereus,* were carried out by using a disc diffusion technique, the highest antibacterial activity was detected against *Pseudomonas aeruginosa* (16.5 mm inhibition zone); and its lowest against *Klebsiella pneumonia* (11.95mm inhibition zone).The antibacterial activity of the synthetic antibiotics, (Ciprofloxacin, Tetracycline Ceftriaxone, Chloramphenicol and Gentamycin, were tested by the disc diffusion method, and by measuring zones of inhibition, shows that there were significant differences, among all antibiotic the highest activity of antibiotic against bacteria was due to the action of ciprofloxacin (22.12 mm inhibition zone) and the lowest activity was due to the action of Ceftriaxone (10.8mm inhibition zone), among the bacteria, the highest inhibition zone by antibiotic is *Salmonella typhimurium*(17.8 mm inhibition zone), the lowest inhibition zone by antibiotic is *Klebsiella pneumonia* (10.96mm inhibition zone), there was no significant different between antibiotic and the volatile oil of *Ocimmum basilicum* in this study.

Introduction

Ocimum basilicum is widely distributed in tropical and warm temperate regions of the world. It is also named as Rehan. Basil (Ocimum spp.), belonging to the family Lamiaceae perennial shrub which grows in several regions all over the world[1, 2] Basil is an aromatic and herbaceous plant that is annual and perennial [3]. Basil has a characteristic odor and sharp taste. The chief constituents include chavicol methyl ether or estragole, linalool and eugenol [4].The studies in the literature suggest linalool as the main active agent responsible for antibacterial activity [5], utilized in food as a flavoring agent, and in perfumery and medical Basil cures headache, improves digestion and is also good for toothache, and snake bite, also is effective in treatment of stomach problems, fever, cough, gout and given internally to treat cystitis, nephritis and in internal piles. Infusion of basil seed is used to treat gonorrhea, chronic diarrhea and dysentery [6, 7, 8]. Plant is also used to keep away insects and snakes [9]. However, recently the potential uses of *Ocimum basilicum* volatile oil, particularly as antimicrobial and antioxidant agents, a plant with extraordinary medicinal properties and contains several antioxidant compounds [10, 11, 12].

Objective

The present study was to identify the volatile Oil Composition of *Ocimum basilicum* (Rehan) leaf extract and antimicrobial activity against human pathogenic bacterial and to compare that with the antimicrobial activity of synthetic antibiotics.

Materials and method

Plant material

The study was executed at the experimental farm of Medicinal and Aromatic Plants Research Institute at Shambat, Sudan (Latitude1540N, Longitude 3232 and 360 m above sea level). The climate is semi-arid with low relative humidity and daily mean air temperature ranging from 25 to 40ºC in summer, and 15 to 21ºC in winter. *Ocimum basilicum* cultivated in the Demonstration Farms of Medicinal And Aromatic Plant Research Institute at Shambat (Sudan). The Plant samples were identified in the department of plant taxonomy in the same institute, collected dried and kept in carton bags for extraction.

Microorganism

All the microorganisms used in this work were obtained from the Stak Laboratory(Khartoum) Sudan. Bacterial identification was carried out by conventional biochemical methods according to the standard microbiological techniques These microbes were *Staphylococcus aureus, Escherichia coli, Pseudomona aeruginosa, Salmonella typhimurium, Klebsiella pneumonia* and *Bacillus cereus*

Extraction of Volatile Oil

The volatile oil from *Ocimum basilicum* plants was extracted hydro distillation using Clevenger-type apparatus according to the method described, dried leaves of the plants (about 100 g) were cut into small pieces and subjected to hydro distillation for 5 h using a Clevenger type apparatus; the oils obtained were dried over anhydrous sodium sulphate. [13]

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Identification of components

For the identification of the components, analytical gas chromatography (GC) was performed using DELSI 121 C apparatus fitted with a flame ionization detector and a CP WAX 51 fused silica column (25 m × 0.25 mm i.d., 0.25 µm film thickness). Temperature was kept at 50°C for 5 min and programmed to reach 220°C at the rate of 3°C per min. A CP W AX 51fused silica WCOT column (60 m × 0.25 mm i.d., 0.25 µm) for GC/MS was used with helium as carrier gas. For GC/MS, a CPWAX 52 fused silica CB column (50 m × 0.3 mm; 0.25 µm film thickness) was used with helium as carrier gas and coupled to a HP mass spectrometer: ionization energy 70 eV. Temperature programming was from 50-240°C at the rate of 3°C/min. The samples were injected at the injector temperature of 240°C. The components were identified by their retention times (RT) and mass spectra with those obtained from the authentic samples and/or the MS library the oil was kept tightly in a sample bottle and stored at $4C^{o}$

Microbial Sensitivity Test of the Volatile Oil

The sensitivity test of the volatile oil of *Ocimum basilicum* was carried out using a disc diffusion technique on Muller Hinton agar with little modification. Sterile 5 mm diameter paper disc soaked with the volatile oil was placed gently on the media, which had been freshly inoculated with each of the organisms. The plates were incubated for 24 hours at 37 C^{o}. The results were recorded by measuring the zone of growth inhibited by the oil [14].

Microbial Sensitivity Test of the antibiotics

To test antibacterial activity of the synthetic antibiotics, standardized discs of Ciprofloxacin (5µg), Gentamycin (10 µg), Ceftriaxone (30µg), Chloramphenicol (10µg), Tetracycline (25µg), were tested by agar disc diffusion method by placing on a streaked Hinton agar plate surface. The antimicrobial activity was also detected by measuring zones of inhibition.

Statistical analysis

Was done according to Duncan Multiple Range Test [15]**.**

Results and Discussion

The dried leaves of *Ocimum basilicum*100 g, produced 8.25%of concentrated volatile oil. Chemical compositions of the Volatile oils of *Ocimum basilicum* L. are given in Table. 1 in the order of the retention times of the constituents. 21 constituents were identified, Methyl eugenol (54.02%), Linalool (21.34%)α-cubebene (5.200%), Limonene (1.01%) nerol (0.875%), epsilon-muurolene(0.831%) α-pinene (0.76%) β-cubebene (0.310%) and β-elemene(0.251%) were found as the major compound, these results were in agree with those obtained in previous studies, were reported that the oil of *Ocimum basilicum* contained (E)-α-bergamotene (3%), thymol (2%). Linalool (45.7%), eugenol (13.4%), methyl eugenol (9.57%), fenchyl alcohol (3.64%) methyl chavicol (87.3%), β-car yophyllene (2.4%), α-pinene (1.0%), β-pinene (0.8%), limonene (0.5%) and camphene (02%) as the major compounds of the volatile oil (2,5,16,17,18). Table 2, show that the antibacterial activity of the volatile oil of *Ocimum basilicum* against different Six pathogenic organisms *Staphylococcus aureus*, *Escherichia coli*, *Pseudomona aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumonia* and *Bacillus cereus*, (the highest concentration of the volatile oil of the leaf extract is (100 mg/ml) and the lowest one is (12.5 mg/ml) there was significant differences among bacteria, the highest antibacterial activity was detected against *Pseudomona aeruginosa* (16.5 mm) and the lowest inhibition zone *Klebsiella pneumonia* (11.95mm), these results conformity with those obtained in previous studies [19,20] who also studied the antimicrobial activities of *Ocimum basilicum* leaf extract against eight bacterial strains using the disc diffusion method, who found that higher antimicrobial activity against the tested Gram positive microorganisms. Also reported that there was an effect of ethanolic extract of Rehan leaves, the inhibition zones and the growth of bacteria was completely inhibited at the highest concentration of the extract. Table3. exhibits the mean zones of inhibition (in mm) for the different synthetic antibiotic shows that there was significant differences among them, highest activity of antibiotic against bacteria was due to the action of ciprofloxacin (22.12 mm) among all antibiotic and the lowest activity was due to the action of Ceftriaxone (10.8mm), the highest inhibition zone among the bacteria by antibiotic is *Salmonella typhimurium*(17.8 mm) and the lower inhibition zone is *Klebsiella pneumonia* (10.96 mm), there was no significant different between antibiotic and volatile oil of *Ocimum basilicum* in this study.

Table 1. Chemical composition of *Ocimum basilicum* L. volatile oil.

No	RT	Compound	Concentration%
1	8.70	α-pinene	0.76
2	9.90	Benzaldehyde	0.002
3	10.26	Sabinene	0.003
4	10.40	Myrcene	0.007
5	12.21	p-cymene	0.007
6	12.31	Limonene	1.01
7	12.48	Eucalyptol	0.002
8	12.97	cis-beta-ocimene	0.009
9	15.10	Linalool	21.34
10	16.15	menth-2-en-1-ol	0.002
11	17.05	Pinocarvone	0.003
12	18.10	terpinen-4-ol	0.009
13	18.50	Nerol	0.875
14	19.34	Neral	0.009
15	20.66	Carvacrol	0.030
16	22.09	α-cubebene	5.200
17	22.95	geranyl acetate	0.088
18	23.29	β-elemene	0.251
19	23.45	β-cubebene	0.310
20	23.56	methyl eugenol	54.02
21	24.45	epsilon-muurolene	0.831

Table 2. Mean zones of inhibition (in mm) for different concentrations of volatile oil of *Ocimum basilicum* leaf extract.

Microorganism	Concentration of the Volatile Oil of leaf Extract from of *Ocimum Basilicum* (µg/disc)				
	100	50	25	12.5	Mean Organism
Salmonella typhimurium	17	15	13	11	14 c
Staphylococcus aureus	15	13.6	12	10	12.66 d
Pseudomonas aeruginosa	28	10	14	14	16.5 a
Escherichia coli	16.9	13	15	12	14.2 c
Klebsiella pneumonia	15.8	12	9	11	11.95 d
Bacillus Cereus	23	15	10	13	15.3 b
Mean volatile oil concentration	19.3 a	13.1 b	12.2 c	11.8 c	

Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

Table 3. Mean zones of inhibition (in mm) for different antibiotics.

Bacterial test strains (No. tested)	Antibiotics concentration in (µg/disc)					
	CIP	G	Chlora	Tetr	CEF	Mean organism
Salmonella typhimurium	27	17	20	11	14	17.8 a
Staphylococcus aureus	22	10.5	21	14	11	15.7 b
Pseudomonas aeruginosa	28	10	14	14	16	16.4 b
Escherichia coli	16.9	13	15	10	11	13.18 c
Klebsiella pneumonia	15.8	12	9	11	7	10.96 d
Bacillus cereus	23 a	15 b	10 c	13 b	6 d	13.4 c
Mean antibiotic	22.12a	12.9c	14.8 b	12.2c	10.8 d	

CIP = Ciprofloxacin (5 µg) G, = Gentamycin (10 µg), CEF = Ceftriaxone (30µg) Chlora =Chloramphenicol, (10µg), Tetr = Tetra cycline (25µg).

Means followed by similar letter are not significantly different at 0.05 level of probability according to Duncan's Multiple Range Test.

Conclusion

This work comes to conclude that the volatile oil of *Ocimum basilicum* L leaf extract had potent antibacterial activity against pathogen bacteria, and have no different between it and synthetic antibiotic in the activity against microorganism, and it is recommended to isolate and separate the bioactive compounds responsible for this antibacterial activity.

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