Essential oil composition and antibacterial effects of Ziziphora clinopodioides(LAM)

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Abstract

Aims: To determine antibacterial effects of Iranian native *Ziziphora clinopodioides* and chemical composition of its essential oil. Methods: Aerial parts of the plant were extracted by methanol (70% in water) and its essential oil was obtained by hydro-distillation procedure. The isolated materials were tested against two Gram positive and three Gram negative bacterial species by using agar diffusion method. Also MICs and MBCs of extract and essence were determined using macro broth dilution method. Compounds of essential oil separated and identified by GC and GC/MS analysis.

Results: The growth of Gram positive organisms (S. aureus and S. pyogenes) were inhibited by methanolic extract at a concentration of 25mg/disk, but it did not inhibit growth of any Gram negative species. The oil halted the growth of all tested Gram positive and Gram negative organisms, with highest effect on S. typhimurium (MIC and MBC = 225mcg/ml). Gas chromatographic analysis revealed 22 different compounds in the essential oil which five of them comprise more than 73% of oil and Pulegon is the highest one. Conclusion: The results suggest that *Ziziphora clinopodioides* has potent antibacterial effects on some bacterial species especially Salmonella typhymurium.

Introduction:

Conventional drugs usually provide effective antibiotic therapy for bacterial infections but there is an increasing problem of antibiotic resistance and a continuing need for new solutions [1]. The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [2]. While some of the plants used on the basis of their reputed antimicrobial properties have well documented *in vitro* activity, there are few published data for many others. *Ziziphora clinopodioides* Lam, which belongs to the Labiatae family and grows wild in many parts of Iran, is one of that plants [3]. This plant is used as stomachic, antifever, anti-inflammatory, sedative and flavoring agent in Iranian Traditional Medicine [4,5]. The antibacterial activity of methanol extract and essential oil of *Ziziphora clinopodioides* and chemical composition of its oil were determined in this study.

Material and Methods:

Plant: Aerial parts of *Ziziphora clinopodioides* (Lamiaceae) collected in June 2003 from southern Alborz Mountains (Lavasan and Laar area of Tehran). The plant was identified at Department of Pharmacognosy, Shahid Beheshti University of Medical Sciences, and a voucher specimen is kept.

Preparation of extracts: Air-dried, powdered plant material (100 g) was successively extracted (three times) for 96 h at room temperature with methanol (70% solution in distilled water). The solvent (methanol) was rotary evaporated after filtration of combined extracts and the extract was freeze-dried.

Isolation of the essential oil: 100 g of dried plant material was subjected to hydrodistillation for 3 h using a modified Unger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulphate and 2 µl was used for GC-MS measurements.

Gas chromatography-mass spectrometry: The analyses of the volatile compounds were carried out using a Shimadzu GC system (9-A-Shimadzu) and GC-MS system (Varian 3400). The fused silica BD-5 polyethylene glycol column (30 m × 0.2 mm, 0.2 µm film thickness) was directly coupled to the mass spectrometer. The carrier gas was helium (1 mL/min) and the program

used was 4 min isothermal at 70 °C, followed by 70–180 °C at a rate of 4 °C/min, then held at 180 °C for 10 min; the injection port temperature was 260 °C. Individual constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds.

Test microorganisms: The antimicrobial activity of essential oil and methanol extract were evaluated using a range which included two Gram-positive bacteria: Staphylococcus aureus (ATCC25923), Streptococcus pyogenes (PTCC1470), and four Gram negative species: Escherichia coli (ATCC25922), Salmonella typhimurium (PTCC1609), Klebsiella pneumoniae(PTCC1053), and Pseudomonas aeruginosa(ATCC27853).

In vitro antimicrobial tests: For preliminary susceptibility tests, standard disk diffusion method was used. Then MICs were determined by the broth macrodilution method in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines (2003) but with the following modifications: the MIC was defined as the lowest concentration that yielded no visible growth after 24 h of incubation at 35 °C and the minimum bactericidal concentration (MBC) was the point at which 0.1% or less of the initial inoculum survived. Aliquots of the oil under investigation was dissolved in 0.5% dimethylsulphoxide (DMSO) to give a solution with in the concentration range 0.03%–4.0% (w/v). In case of methanol extract the concentration range was 0.1-25 (mg/ml). The broth cultures of selected organisms were prepared by inoculating Muller-Hinton broth (MHB) with 1–2 colonies from a blood agar plate and incubating for 18 h at 37 °C with shaking. Serial doubling dilutions of oil and extract were prepared in 15x100 mm tubes and incubated aerobically at 35 °C for 24 h. To confirm MIC and to establish MBC, aliquots of 10 µL were removed from each tube; spot inoculates onto Muller-Hinton agar (MHA). After aerobic incubation at 35 °C overnight, the number of surviving organisms was determined. Each test was performed in triplicate and the results analyzed.

RESULTS AND DISCUSSION:

The essential oil isolated was obtained in yield of 0.96% (w/w) based on dried material. Twenty two compounds were identified in the oil of *Z. clinopodioides*, representing 97% of the total oil. These are detailed in Table 1. The oil is characterized by a high content of pulegone (29.3%). Other important compounds were p-mentha-3-en-8-ol (19.02%), neo-menthol (11.58%), piperitenone (9.43%), and 1,8-cineole (4.48%). The essential oil also contained smaller percentages of menthone, piperitone, spathulenol, carvone hydrate, and isomenthol. A review of the published literature reveals that the

composition of *Z. clinopodioides* oils from different regions shows largs similarity in the major components, but relative concentrations have some differences. It is probable that environmental conditions have a significant influence in the relative amounts of essential oil components. Also pulegone is the major component of other species of Ziziphora (*Z. hispanica, Z. brevicalyx, Z. tenuior*) in the published literature [6-7]. Pulegone has predominant antibacterial and antifungal activity, and it is very effective on Salmonella species but it is a highly toxic compound for liver [8]. The antimicrobial activity of *Z. clinopodioides* essential oil and methanol extract were evaluated as previously described. The results presented in Tables 2 and 3 show that this oil exhibited antimicrobial activity to varying degrees against all strains tested.The essential oil showed the highest antimicrobial activity with MIC values ranging from 0.25% to 2% and MBC values from 0.25% to 2%. The maximum activity of oil was observed against *Salmonella typhimurium* with significant growth inhibition observed at a concentration as low as 225mcg/ml.

The methanol extract of *Z. clinopodioides* was also found to inhibit the growth of *S. aureus* and *S. pyogenes*, and the MIC and MBC of the extract against both species was 15 mg/ml and 31 mg/ml. It did not inhibit the growth of any of the tested Gram negative species.

We conclude that *Ziziphora clinopodioides* has potent antibacterial activity aganist some Gram positive and Gram negative bacterial species especially Salmonella typhymurium and it can be used as a complementary therapy, however, it is most important that to consider the toxic level of the plant or isolated materials.

Table1. Chemical composition of *Ziziphora clinopodioides* essential oil (GC-MS analysis)

Compound	Relative%
α-pinene	0.36
camphene	0.38
sabinene	0.24
β-pinene	0.58
p-cymene	0.47
limonene	0.44
1,8-cineole	4.48
p-mentha-3,8-diene	0.34
p-menth-3-en-8-ol	19.02
menthone	3.62
neo-menthol	11.58
menthol	1.06
isomenthol	1.78
neoisomenthol	0.73
cis-pulegol	1.04
pulegone	29.28
piperitone	2.43
bronylacetate	1.17
piperitenone	9.43
β bourbonene	0.95
carvone hydrate	1.69
spathulenol	2.35

Table2. Antimicrobial activity of *Ziziphora clinopodioides* essential oil and methanol extract

Microorganism	essential oil		methanol extract	
	MIC Concentration (v/v %) (mcg/ml)	MBC Concentration (v/v %) (mcg/ml)	MIC Concentration (mcg/ml)	MBC Concentration (mcg/ml)
Staphylococcus aureus ATCC25923	1.0% 900	1.0% 900	15000	30000
Streptococcus pyogenes PTCC1470	0.5% 450	0.5% 450	15000	30000
Escherichia coli ATCC25922	2.0% 1800	2.0% 1800	-	-
Salmonella typhimurium PTCC1609	0.25% 225	0.25% 225	-	-
Klebsiella pneumoniae PTCC1053	1.0% 900	1.0% 900	-	-
Pseudomonas aeruginosaATCC27853	2.0% 1800	2.0% 1800	-	-

"–" "No growth inhibition, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

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