Antimicrobial activity of volatile oil and fixed oil extracted from *Anchusa strigosa* Lab.

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Abstract

The aim of this study was to extract the volatile and fixed oils from the dry flowers of *Anchusa strigosa* Lab. By Soxhlet extraction method and steam distillation, and to examine the antibacterial effects of these oils on different bacterial strains.

The volatile oils exhibited potent antibacterial activity against most of the organisms examined , than the fixed oils. The results reported in this paper indicate that volatile and fixed oils of dry flowers of *Anchuse strigosa* Lab. Can be considered as a potential antimicrobial agent for the treatment of some microbial infections.

Introduction:

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind . The search for eternal health and longevity and for remedies to relieve pain and discomfort drove prehistoric man to use many plants.(1)

Today , there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. Nature has bestowed upon us a very rich botanical wealth an a large number of divorse types of plants grow wild in different parts of IRAQ. One of these important medicinal Iraqi herbs, was the dried flower of *Anchusa strigosa* Lab. (Boraginaceae), which is called (Lisan-el-thor) in Arabic.(2)

In previous papers, we reported on the antibacterial activity of the water –extract and a pure proteins isolated from the water-extracted dry flowers of this plant a gainst different strains of bacteria(3,4). Ahmed *et al*.(1998) has reported that the aquous root extract of *Anchusa strigosa* Lab. Was effective against gastric – ethanol – induced ulcer in laboratory animals, and that activity was related to the presence of Anchusin (a red-brown resinoids coloring material).(5) Later six new pyrrolizidine alkaloids and glycoside compounds have been isolated from *Anchusa strigosa* roots, and its antifeedant activity was investigated.(6)

Now in this work and as a part of our effort to identify the substances responsible for the pharmacological activities attributed to *Anchusa strigosa* Lab., which utilized in Iraq in popular medicine, we have studied the antibacterial activity of the volatile (essential) and fixed oils extracted from the dry flowers of *Anchusa strigosa* Lab.

Eperimental:

Extraction of volatile and fixed oils(7):

10 gm. of the dry flowers of *Anchusa strigosa* Lab. Was extracted with n-Hexane (100ml), using Soxhlet apparatus for 24 hours. The organic solvent was evaporated by rotary evaporator, and the residue was considered as percentage of total lipids. Then the volatile oils were separated from the fixed oils in the total lipids extracted above by steam distillation with using of ethanol as a solvent.(7)

Assay for antibacterial activity(8):

The antibacterial activity of the volatile and fixed oils separated above against different bacterial strains was investigated off by a disc diffusion method. Petri plates containing 20ml of nutrient agar medium were sealed with old cultures of the bacterial strains.

The volatile and fixed oils extrated fractions were tested in different concentrations (10-500µl /ml.), applying 0.1ml of each sample to sterile filter paper discs (5mm. in diameter) and placed on the surface of the medium. The inoculums size was adjusted so as to deliver a a a final inoculums approximately 5×10^7 colony forming units (CFU/ml.). Incubation was made at 37ºc for 24 hours. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed around the disc. For each bacterial strain controls were maintained in which pure solvent (ethanol) were used instead of the extract, then the control zones were subtracted from the test zones.

Results and discussion:

The total lipids of dry flowers of *Anchusa strigosa* Lab. Were extracted by continuous Soxhlet apparatus, and its percent was 4.4% . While the volatile (essential) oils and fixed oils were separated by steam distillation , and their percent were 26.1% and 52.8% of total lipids respectively , as shown in Table(1).

The *in vitro* antibacterial test of the volatile and fixed oils resulted in a range of growth inhibition pattern against the used bacterial strains , as shown in Tables (2)and (3), in comparison with a standard antibiotics shown in Table (4).These data revealed that the volatile oil of *Anchusa strigosa* Lab. exhibits potent antibacterial activity against both Gram positive and Gram negative bacteria, especially in a high concentrations (200 and 500µgm /ml). It is noteworthy in particular its effect against *Pseudomouos aeruginsa*, *Proteus sp.* and *Streptococcus faecalis* , which was comparable with Cephalexin (300mg.), Nalidixic acid (30mg.) and Nitrofurantion (300mg.) effects and better than other antibiotic effects , as shown in Tabe(4). On the other hand , the fixed oil showed good activity a gainst *Klebsiella sp.*, *Proteus sp.* and *Pseudomonos aeruginosa* especially at higher conc. (500µg. /ml).

However, the volatile oil showed greater inhibitory activity when compared to fixed oil. The biological and pharmacological activity of the medicinal plants is related to presence of different materials such as: alkaloids, glycosides, proteins and volatile oils; e.g. the antibacterial activity of *Tocoma stuns* L. is related to the

volatile oil (Laphacol)[10], *Mentha spicata* L. is due to volatile oil (Carvone)[11]. *Thymus capitatus* L. is related to Carvone as well[12].*Foeniculum vulgare* L. and *Nigella sative* L. is due to volatile oil (Antethol)[13],[14]. Now a days the use of volatile oil (essential oil) for the purpose of affecting a person's mood and health represents alternative medicine called (Aromatherapy)[15], and these result was in good agreement with literature in which the volatile oils from other medicinal plants of the western region of India showed maximum antibacterial activity against different bacterial strains[9].

Hence, the investigating of the antibacterial effect of volatile and fixed oils extracted , is in consitent with our ealier observation [3],[4], in which the antibacterial activity of water extract and a pure proteins isolated from the dry flowers of *Anchusa strigosa* Lab. , which can be selected for further analysis and can be used to discover bioactive natural products that may serve as leads in the development of new pharmacenticals that address unmet therapeutic needs. Such screening of various natural organic compound and identification of active a gents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

Table (1):The percentage of total lipids , volatile (essential) and fixed oils extracted from the dry flowers of *Anchusa stigosa* Lab.

Total lipids % of dry wt.	Volatile oil% of total lipids	Fixed oil% of total lipids
4.4%	26.1%	52.8%

Table (2):The antibacterial activity of volatile oil extracted from dry flowers of *Anchusa stigosa* Lab., as inhibition zone(mm.)

Bacterial strain	Volatile oil concentration				
	10µg/ml	50µg/ml	100µg/ml	200µg/ml	500µg/ml
Escherichia coli (Gr.-ve bacilli)	1	3	6	8	9
Enterobacter sp. (Gr.-ve bacilli)	0	0	4	5	8
Klebsiella sp. (Gr.-ve capsule fomring)	0	2	5	7	8
Proteus sp. (Gr.-ve bacilli)	5	7	12	15	20
Pseudomonas aeruginosa (Gr.-ve bacilli)	3	9	15	17	25
Bacillus subtilis (Gr.+ ve bacilli spore forming)	0	3	7	8	10
Streptococcus faecalis (Gr.+ve bacilli)	1	5	7	9	15
Streptococcus viridance (Gr.+ve cocci)	0	0	3	6	8
Staphyloccus epidermidis (Gr.+ve cocci)	1	3	7	8	10
Staphylococcus aureus (Gr.+ve cocci)	0	1	5	9	10

Table (3):The antibacterial activity of fixed oil extracted from dry flowers of *Anchusa stigosa* Lab., as inhibition zone(mm.)

Bacterial strain	Fixed oil concentration				
	10µg/ml	50µg/ml	100µg/ml	200µg/ml	500µg/ml
Escherichia coli (Gr.-ve bacilli)	2	4	4	5	4
Enterobacter sp. (Gr.-ve bacilli)	0	0	2	3	7
Klebsiella sp. (Gr.-ve capsule forming)	3	5	6	6	12
Proteus sp. (Gr.-ve bacilli)	0	4	8	10	12
Pseudomonas aeruginosa (Gr.-ve bacilli)	0	1	3	7	10
Bacillus subtilis (Gr.+ ve bacilli spore forming)	0	0	2	3	6
Streptococcus faecalis (Gr.+ve bacilli)	2	3	5	5	8
Streptococcus viridance (Gr.+ve cocci)	0	0	2	5	7
Staphylococcus epidermidis (Gr.+ve cocci)	2	3	5	8	8
Staphylococcus aureus (Gr.+ve cocci)	0	2	3	5	7

Table (4):The effect of standard antibiotics against bacterial growth, as inhibition zone(mm.)

Bacterial strain	Antibiotic									
	E	K	TE	CP	NA	F	C	CR	AMP	P
Escherichia coli	11	10	5	9	13	11	12	10	6	8
Enterobacter sp.	7	11	7	10	10	10	13	2	5	7
Klebsiella sp.	8	9	16	14	12	13	11	10	7	5
Proteus sp.	11	12	11	15	18	12	10	12	11	10
Ps. Aeruginosa	10	8	6	12	13	17	16	14	12	6
Bacillus sp.	6	9	13	12	11	9	10	8	6	3
Staph. Aureus	4	11	8	17	7	10	9	10	9	7
Staph. Epidermidis	7	10	4	16	9	6	8	6	4	5
Strept. sp.	12	9	10	16	8	12	9	6	10	7

P:Pencillin (10 units); AMP:Ampicillin (10mg) ; CR:Carbenicillin (100mg) ;
C:Chloramphenicol (30mg) ; F:Nitrofurantion (300mg) ; NA:Nalidixic acid (30mg) ;
CP:Cephalexin (30mg) ; TE:Tetracycline (30mg) ; K:Kanamycin (30mg) ;
E:Erythromycin (15mg)

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الفعالية البايولوجية للزيوت الطيارة والثابتة المستخلصة من ورد لسان الثور *Anchusa strigosa* Lab.

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الملخص

تضمن البحث فصل الزيوت الطيارة والثابتة من الدهون الكلية المستخلصة من ورد لسان الثور باستخدام طريقتي الاستخلاص بـ Soxhlet والتقطير البخاري، ودراسة فعاليتها البايولوجية المضادة للعديد من البكتريا. وقد اثبتت الزيوت الطيارة تأثيرها التثبيطي العالي مقارنة بالزيوت الثابتة ضد النمو البكتيري للعديد من الاصناف الموجبة والسالبة والذي يشابه تأثير العديد من المضادات الحيوية ضد النمو البكتيري.