



Chemical Composition and Antibacterial Activity of the Essential Oil from *Erinacea anthyllis* Link (Fabaceae)

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ABSTRACT

Erinacea species is used in traditional medicine to treat rheumatic diseases. The purpose of this study was to determine for the first time, the chemical composition and antibacterial activity of essential oil from the fresh aerial parts of *Erinacea anthyllis* belonging to the Fabaceae family. The steam distilled oil was analyzed using gas chromatography techniques (GC-FID and GC-MS) using two different stationary phase columns (polar and non polar). Furthermore, antibacterial activity against various gram-positive and negative bacteria was determined by disk diffusion and microdilution. Altogether 44 volatile substances, representing around 98.22% of the essential oil was identified. The major constituents were phytol (9.26%), tricosane (8.62%). Moreover, essential oil exhibit effect on all bacterial strains. The obtained inhibition zone ranged from 7 mm to 21mm with a highest inhibition zone recorded for *Bacillus Sp* (21mm). *Erinacea anthyllis* essential oil has good antimicrobial activity against all tested pathogenic bacteria and may be used as a natural antimicrobial agent in the treatment of many infectious diseases.

Keywords: *Erinacea anthyllis*; antibacterial; essential oil; Batna; Algeria

Introduction

The Fabaceae family, known as Leguminosae, is a large and diverse family of flowering plants that includes over 19000 species. It is one of the most economically important plant families, as many species are used for food, fodder, fuel and medicinal purposes.¹⁻²

Several species within the Fabaceae family have been found to possess antibacterial properties against a range of pathogenic bacteria, including *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Streptococcus pyogenes*.³⁻⁷ The genus *Erinacea* belongs to the legume family, the tribe Genisteae, while is represented by a single species called *Erinacea anthyllis* Link or *Erinacea pungens* Boiss.⁸ *Erinacea anthyllis*, also known as blue broom, hedgehog plant, or rushy kidney vetch, is a species of flowering plant. It is a dwarf, spiny shrub that grows under 30cm tall and has erect branches terminating in sharp spines. The leaves are inconspicuous and the flowers are blue and mauve. It is native to Spain and Algeria. Moreover, it is a rare and choice spiny legume.⁹ *E. anthyllis* is used in traditional medicine in Algeria to treat rheumatic diseases and as a honey source in the siroua region of Morocco.¹⁰ *Erinacea anthyllis* contains several secondary metabolites including polyphenols, flavonoids and steroids. Two new prenylated isoflavonoids named ErinasonA and ErinasonB were isolated from the plant along with 19 known secondary metabolites. These two isoprenylated isoflavonoids have been found to have antioxidant and antibacterial activities.¹¹ There is no information available in the provided search results about essential oil of *E. anthyllis*.

The search results mainly provide information about the plant's taxonomy, morphology, habitat, and chemical composition. Therefore, the chemical composition of the essential oil extracted from the aerial parts of *E. anthyllis* was investigated in this study. Furthermore, the antibacterial capacity was evaluated against four common pathogens.

Materials and Methods

Plant collection and identification

Stems, leaves, flowers of *Erinacea anthyllis* Link. were collected during the flowering stage in May 2022 from Batna, eastern of Algeria. Plant materials were authentically identified by Prof. Khellaf REBBAS, Department of Biological Sciences, University of M'sila. A voucher specimen (Number N°VAREAT5/22) was deposited at the Herbarium of VARENBIOMOL unit, Constantine, Algeria.

Extraction of essential oil

The fresh aerial part of *Erinacea anthyllis* (150 g) was subjected to steam distillation in a Kaiser Lang apparatus for three hours. The resulting oil (750 mg) was collected and dried over anhydrous sodium sulphate and kept at 4 °C until analysis. Oil yield is calculated based on plant weight.

GC-FID and GC-MS analysis

GC-FID essential oil components were analysed using a SCHIMADZU GC-2010 chromatograph equipped with Rxi-5ms capillary column (30 m * 0.25 mm, film thickness 0.25 µm). Helium was the carrier gas, at a flow rate of 1.44 mL/min. The oven temperature was maintained at 45°C for 10 min and then increased to 180°C at a rate of 3°C/min and maintained at 180°C for 5 min, then to 280°C at a rate of 5°C/min and maintained at 280°C for 5 min and finally to 330°C at a rate of 10°C/min for 2 min. Injector and detector (FID) temperatures were set at 330 °C. Diluted samples (in dichloromethane) of 1 µL were injected in the split/splitless (30:1 split) mode GC-MS analysis was performed using GCMS-QP2010. The Mass selective detector was equipped with a capillary column Rxi-5ms capillary column (30 m * 0.25 mm, film thickness 0.25 µm).

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Helium was the carrier gas, at a flow rate of 1.44 mL/min. The oven temperature was maintained at 45°C for 10 min and then increased to 180°C at a rate of 3°C/min and maintained at 180°C for 5 min, then to 280°C at a rate of 5°C/min and maintained at 280°C for 5 min and finally to 330°C at a rate of 10°C/min for 2 min. For GC-MS detection, an electron ionization system with ionization energy of 70 eV, was used. Injector temperature was: 330°C. Diluted sample (in dichloromethane) of 1 µL were injected in the split/splitless (30:1 split) mode. Identification of oil components issued from capillary column was accomplished based on comparison of their retention index to those of literature and also comparison of their mass fragmentation patterns with those of databases.¹²

Antimicrobial assay

E. anthyllis oil has been evaluated against four common pathogens, *Bacillus*, *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), and *Pseudomonas aeruginosa* (ATCC27853). To study antibacterial activity, sterile Muller Hinton agar medium was prepared in petri dishes, and yeast starch agar was inoculated separately into the medium alone. Aseptically prepare four wells (6 mm in diameter) and add 80 µL of each extract prepared in DMSO to the wells. Plates were incubated at 37°C for 24 hours before the zone of inhibition was measured. At the end of the incubation period, the zone of inhibition was measured.¹³

Minimum inhibitory concentration determinations (MIC)

The minimal inhibitory concentration (MIC), is defined as the lowest dose of antibiotic that inhibits observable bacterial growth after 24 hours of incubation. In this regard, the essential oil of *E. anthyllis* was diluted decimally with dimethyl sulfoxide (DMSO) and 10 µl of each dilution was pipetted into a sterilize paper disc (ø6 mm) placed in a petri dish. Petri dishes containing previously inoculated Mueller-Hinton agar containing the culture of the bacteria to be tested, a negative control is also included in the test. The possible activity of

the solvent against the bacteria to be tested is checked using a paper disc soaked in DMSO. The dishes were then incubated at 37°C for 24 hours.¹⁴

Results and Discussion

Chemical constituents of essential oil

The steam distillation of *E. anthyllis* aerial part yielded a yellow essential oil with perfumery odour (0.35%, w/w). Identification of compounds was carried out using Target, Wiley 8, FFNSC1.2, NIST11, and Adams data bases.¹² These identifications were confirmed by linear retention indices (RI). We detected 44 compounds representing 98.22% of the total composition (Table 1, Figure 1 and Figure 2). The main compounds were phytol (9.26%), tricosane (8.62%), β-thujone (7.66%), Artemisia ketone (6.64%), α-Murolene (5.74%), 2-hexadecanone 6,10,14 trimethyl (6.22%) and Hexacosane (5.56%). Phytol and tricosane are two chemical compounds that are found in many essential oils, including some varieties of lavender, lemongrass, and peppermint. Moreover, β-thujone is another compound that is found in some Fabaceae essential oils, including wormwood and sage.¹⁵

Phytol is a diterpene alcohol that is believed to have anti-inflammatory and antioxidant properties, and it is often used in the fragrance industry.¹⁶ Tricosane is a straight chain hydrocarbon that is commonly found in plant waxes and has been used in some skincare products.¹⁷ β-thujone is a monoterpene that is found in a variety of plants including sage. It is known for its psychoactive properties and has been used in some traditional medicines.¹⁸

E. anthyllis essential oils contain a complex mixture of constituents and thus have various antimicrobial properties, the bulk of this effect appears to come from oxygenated terpenoids, especially phenolic terpenes, phenylpropanes, and alcohols.

Table 1: Chemical composition of *Erinacea anthyllis*. essential oils from Batna (Algeria)

Peak no.	Tr	RI	Components	%
01	21.102	1026	Eucalyptol	0.92
02	23.172	1056	Artemisia ketone	6.64
03	23.397	1065	Cis thujanol	0.64
04	24.515	1079	Artemisia alcohol	0.48
05	25.661	1062	β thujone	7.66
06	26.299	1125	α thujone	0.88
07	27.532	1139	Trans-Pinocarveol	0.48
08	27.822	1141	Camphor	0.92
09	28.884	1164	Pinocarvone	0.48
10	29.525	1172	3-pinanone	0.48
11	39.142	1361	Nerolacetate	0.28
12	39.725	1374	α -copaene	0.60
13	41.144	1402	Lauraldehyde	0.48
14	44.192	1477	β Chamigrene	1.26
15	44.603	1487	E,β Ionone	1.30
16	45.273	1500	α- Murolene	5.74
17	45.431	1504	β-Himachalene	0.4
18	46.234	1523	δ- Cadinene	1.0
19	48.517	1578	Spathulenol	1.24
20	48.764	1583	Caryophyllene oxide	5.10
21	49.110	1592	viridiflorol	1.98

22	49.220	1612	Cetane	0.9
23	49.602	1619	Cubenol (1,10-di-epi)	1.46
24	50.551	1630	Muurola-4,10(14)-dien-1-Beta-ol	1.12
25	51.580	1685	Cedranol (5-neo)	0.56
26	53.116	1700	heptadecane	0.56
27	55.653	1784	2,3-dimethoxy naphtalene	0.6
28	56.982	1800	octadecane	0.62
29	59.064	1854	2-hexadecanone,6,10,14-trimethyl	6.22
30	61.694	1900	Nonadecane	1.18
31	65.451	2000	Eicosane	0.96
32	67.974	2090	Cosyl alcohol	5.10
33	68.422	2100	Heneicosane	4.5
34	68.807	2106	phytol	9.26
35	69.820	2184	3,7,11,15-tetramethyl-2-octadecen-1-ol	0.48
36	70.915	2200	Docosane	0.6
37	73.120	2300	tricosane	8.62
38	75.90	2400	tetracosane	0.88
39	76.612	2549	1-tetracosanol	0.34
40	76.914	2600	Hexacosane	5.56
41	78.611	2700	Heptacosane	0.44
42	80.218	2800	Octacosane	3.60
43	83.864	3000	triacontane	1.90
44	87.071	3200	Docontane	3.8
Oilyield				
0.35				
Totalidentified				
98.22%				

Tr: Retention time obtained by chromatogram;.RI: Retention Index

Table 2: Antibacterial activity of *Erinacea anthyllis* essential oil

Bacterial strains	Inhibition zone (mm) with different essential oil concentrations				
	Pure oil 1/1	Dilution 1/2	Dilution 1/4	Dilution 1/8	MIC
<i>Escherichia coli</i>	15	7	7	0	1/4
<i>Staphylococcus aureus</i>	15	13	10	7	1/8
<i>Pseudomonas aeruginosa</i>	10	12	8	0	1/4
<i>Bacillus sp</i>	21	7	7	0	1/4

Antimicrobial activity

The antimicrobial potential of *Erinacea anthyllis* essential oil was assessed by the zone of inhibition of bacterial growth. The results in Table 2 demonstrated that essential oils have an effect on all bacterial strains. The zones of inhibition varied from 7mm to 21mm with highest zone recorded with *Bacillus Sp* (21mm). MIC values against *Echerichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus sp* are 1/4, 1/8, 1/4, 1/4 respectively.

Half-dilution of essential oils (Table 2) reduced the growth density of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus*, but tested bacteria were resistant to 1/8 of the essential oil concentration, except *Staphylococcus aureus*. The concentrated oil exhibited high levels of activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus*, with inhibition diameters of 15 mm, 15 mm, 10 mm, and 21 mm, respectively, indicating that these bacteria are very sensitive.

E. anthyllis essential oils had very small CMI values (1/4 and 1/8) for the tested microorganisms. These data are important for treating infections caused by these bacteria: *Staphylococcus aureus* is the leading cause of infection because of its virulence and ability to acquire antimicrobial resistance, causing serious problems for hospitals and health care professionals worldwide problem.¹⁹ *Erinacea anthyllis* essential oil has good antibacterial activity and may be used as a natural antibacterial agent in the treatment of various infectious diseases caused by Gram-positive and Gram-negative bacteria.

Conclusion

For the first time, the chemical composition of *E.anthyllis* essential oil and antibacterial activity were investigated. A total of 44 compounds were identified, accounting for 98.22% of the total oil. Good

antibacterial effect of *Erinacea* essential oil has been observed, indicating its high potential as a new source of antibacterial agent. These effects can be traced back to their chemical constituents, such as phytol, tricosane, β -thujone, and α -Muurolene.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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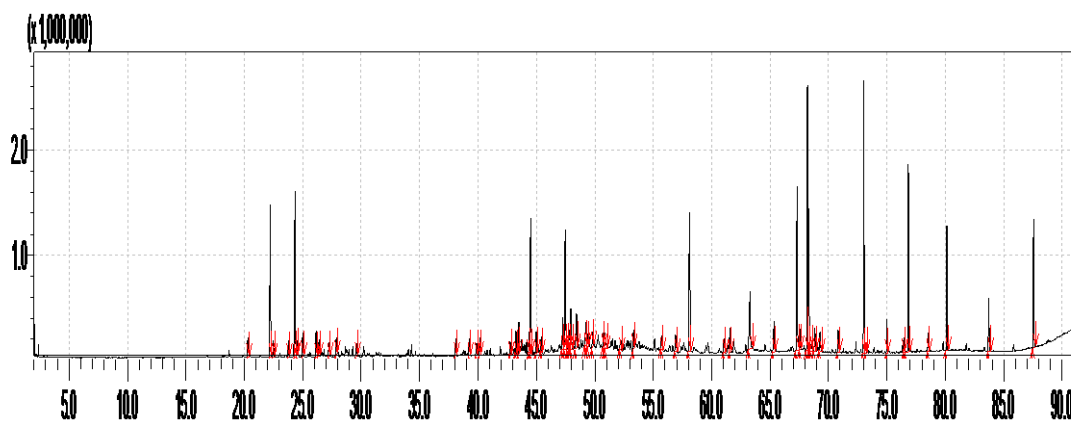


Figure 1:GC-FID chromatogram of *Erinacea* essential oil

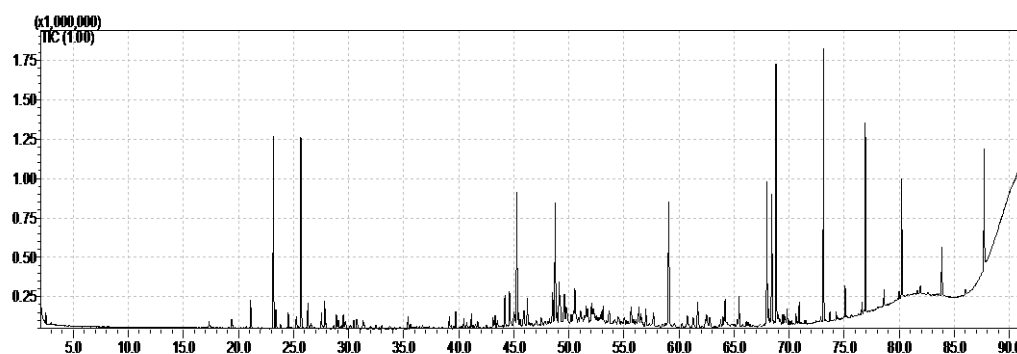


Figure 2:GC-MS chromatogram of *Erinacea* essential oil

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