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GC-MS analysis, antibacterial, antioxidant and cytotoxic activity of *Euphorbia larica* Boiss indigenous to Oman

Abstract

Euphorbia is a diverse genus within the Euphorbiaceae family, comprising over 2,000 species and 600 hybrids. Among these, *Euphorbia larica*—known locally in Arabic as *Isbaq*—is a vital component of Oman's indigenous desert vegetation. This study utilized GC-MS analysis to identify the chemical constituents of *E. larica* leaf oil, revealing 22 distinct compounds. The chemical profile is dominated by monoterpenes (63.6%), followed by sesquiterpenoids (24.16%), triterpenoids (9.2%), and diterpenes (2.8%). Furthermore, the oil underwent biological evaluation using DPPH assays, disc diffusion, and brine shrimp lethality tests, demonstrating significant antioxidant, antibacterial, and cytotoxic activities.

Keywords: *Euphorbia larica*, GC-MS, antioxidant, antibacterial and cytotoxic

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Abbreviations: UV-Vis, Ultraviolet–visible; HPLC, High-performance liquid chromatography; UV, Ultraviolet; DMSO, Dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GC-MS, Gas chromatography-Mass Spectroscopy; EL, Euphorbia larica

Introduction

Euphorbia is a large flowering plant genus in the Euphorbiaceae family with 2000 species.[1]

The wide variety is located in the Middle East, in the Sultanate of Oman, where it expands with in north of the country, and it expanded in all way to a United Arab Emirates. It moreover expands in limited quantities on the Persian Gulf's northern coast in southern Iran. Linked species, grows in Omani and Yemeni boarders.[2]

Euphorbia larica Boiss is widely distributed in the Hajar mountain and Ru'us al-Jibal. It grows in Gravel Plains, mountain slopes at elevation of up to 1300m. The flowering time is from November to April. Groups of light-yellow flowers in terminal and top medial spikes are encircled by yellow-green glands. It bears fruits like spherical brown capsules (1cm across) contain six segments with two seeds each.[3]

Euphorbia larica, also identified as Isbaq in Arabic, is an important and widespread component of Oman's indigenous desert vegetative cover. Latex will have typically been used in ethnobotanical contexts for items like parasite treatment in camels.[4] It has many branches but no leaves, a perennial tree. Although there are leaves, they are very small, and the tree is really between one to three meters. Ethnomedicine research has shown that people treat illness and disease differently depending on their culture.[3]

One of the studies reported quantification of Euphorbia and some phenolic constituents were identified like rutin, hesperidin, hyperoside, quercetin and caffeine by LC-MSMS & GC-MS.[5] Another study published in 2023 on the *Euphorbia larica* Boiss reported α-pinene as major constituent around 27.36% followed by limonene 11.5%. Biological evaluation of organic materials from *E. larica* showed strong antibacterial activity, mild antioxidant and hemolytic activity.[6] Traditionally *E. larica* has a wide range of current and historical uses. In the past and the present, it was used to build roofs, removes thorns from the body. Latex, a milky fluid, was collected and dripped from a cup over the parts of the foot or hand where thorns pierced the flesh. It also treats rheumatoid arthritis pains, treats fractures, treats wounds, and treats scabies in humans. It is also used to catch fish while fishing. In small quantities, the liquid is also used as a laxative to expel worms. However, for oral and topical application the latex should be used cautiously as is known to be toxic, irritating, and can cause skin allergies. *Euphorbia Larica* is used to treat camel parasites bites, boils, burns, skin disorders, gastrointestinal parasites, gonorrhea, headaches, and warts can all be treated with it.[6] Branches are thrown into water to catch fish, which the plant's sap poisons, latex is used as an adhesive to snare birds, and dry leaves are used to thatch homes. Despite being hazardous, latex is used in fishing for fish and birds. Infections caused by camel parasites are also treated with it in small dosages.[7]

Material and methods

Sample collection

Fresh areal parts of *Euphorbia larica* (3 kg) were collected from near Al-hajar mountains Nizwa and washed under running water. The sample was identified by taxonomist from Natural and Medical Sciences Research Centre (NMSRC) at, University of Nizwa, Oman. A voucher specimen number AS/EL/01/2023 has been deposited at NMSRC herbarium.

Extraction of *E. larica* essential oil

Water distillation of *E. larica* (3 kg) areal parts for 6 hrs yielded light yellow volatile oil (1.5 mL, 0.05% v/w). The isolated oil was dried over anhydrous Na_2SO_4 and stored at 4 ºC in the refrigerator till further use.

GC/MS analysis

The areal parts of plants were subjected to steam distillation to isolate the volatile oil. Isolated volatile oil was analysed by GC/MS instrument. Each compound was identified by computer matching with standard spectra. GC/MS "Perkin Elmer Model # Clarus 600

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fitted with a HP-5MS capillary column (30m x 0.25mm i.d. x 0.25µm film thickness).

Antioxidant activity by radical scavenging assay

To determine the antioxidant activity of the isolated volatile oil, a modified Brand-Williams DPPH assay was employed. Oil samples were prepared in hexane at concentrations of 62.5, 125, 250, 500 ug/ml. Absorbance of each sample was measured at 517 nm. The control was prepared in the same way but without adding oil. Radical scavenging activity of the tested concentrations was determined as the percentage inhibition by using the following formula:

$$\%inhibition = \frac{A_o - A_s}{A_o} \times 100\%$$

Where A_o is absorbance of control and A_s is absorbance of test sample.[8]

Antibacterial test

Antibacterial activity was tested by the disc diffusion method. Both gram positive and gram-negative bacteria were used in antibacterial assay. Filter paper discs (Whatman no.1; 6 mm diameter) were impregnated with different concentration of volatile oil prepared in DMSO and standard drug. The discs were placed on Mueller Hinton agar plates and incubated at 37 C, for 24 h. Each test was repeated in triplicate.[9]

Cytotoxic activity

Brine shrimp test

Cytotoxicity assay was done using brine shrimp (Artemia Salina Leach) larvae as indicator organisms.[10]

To hatch the brine shrimp, we prepared artificial seawater by mixing 38 g of sea salt with one liter of distilled water. We then spread 50 mg of cysts in the dark side of a partitioned polyethylene tank. Because brine shrimp larvae are attracted to light, they migrated to the illuminated side of the tank upon hatching (within 24 hours), allowing for easy collection**.**

Brine shrimp lethality test

Stock solution (10mg/ml) of volatile oil in DMSO was diluted to prepare testing concentrations corresponding to 62.5,125, 250, 500 µg/ml. About 3 ml of artificial seawater was added to each vial. A total of 10 shrimp larvae was transferred in each vial and the solution was diluted to 5 ml by using the artificial sea salt. The vials were illuminated and maintained at room temperature for 24 hours. Each experiment was done in triplicate. The numbers of survival were counted, and the percent mortality of the larvae after 24 hours was calculated.

Data analysis

LC_{50} values of the volatile oil were generated by regression analysis using MS Excel.

Results

GC/MS analysis

Results from GC/MS analysis of the volatile oil isolated from *Euphorbia larica* are presented in Table 1. Each compound was identified by computer matching with standard spectra.

Table 1 Chemical constituents of volatile oil from *Euphorbia larica* collected in Oman

#	Compound Name	R.Time (min)	Area	Area%	K.I calc.	K.I
1	*1R*-α-Pinene	8.168	25693512	31.7338	932	922
2	Camphene	8.623	170560	0.210657	946	943
3	β-Pinene	9.472	305041	0.376753	972	970
4	β-Myrcene	9.559	157005	0.193915	974	979
5	Limonene	11.379	403490	0.498346	1028	1020
6	Eucalyptol	11.45	8320541	10.27662	1030	1023
7	Linalool	13.92	275446	0.340201	1100	1082
8	3-Undecyne	14.817	125270	0.15472	1126	1132
9	L-Pinocarveol	15.268	322899	0.398809	1139	1143
10	*cis*-Verbenol	15.499	1395385	1.723426	1145	1148
11	Terpinen-4-ol	16.625	857848	1.059519	1177	1175
12	4-Octene-2,7-diol, 2,7-dimethyl-, Z-	17.108	2151801	2.657668	1191	1207
13	1,11-Dodecadiyne	17.713	114110	0.140936	1209	1210
14	Isobornyl acetate	20.31	190778	0.235628	1286	1278
15	Terpinen-4-ol, acetate	22.419	35952531	44.40461	1352	1332
16	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	23.46	196448	0.242631	1384	1352
17	4-Oxatricyclo [4.3.1.1(3,8)] undecan-5-one	23.507	161808	0.199847	1386	1349
18	Bicyclo [10.1.0] tridec-1-ene	23.736	350093	0.432396	1393	
19	Patchoulane	24.588	125807	0.155383	1421	1393
20	*(5Z)*-5-Pentadecen-7-yne	26.624	495775	0.612327	1488	1538
21	UI	29.442	581122	0.717738	1586	
22	β -Eudesmol	31.337	2618492	3.234073	1654	1644

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Antioxidant activity of *Euphorbia larica*

Table 2 present radical scavenging activity of the volatile oil prepared from *Euphorbia larica.* As can be seen from the Table the DPPH scavenging activity was dose dependant. However, the scavenging activity could not be raised above ~ 83% by increasing the dose to values greater than 500 µg/ml as shown in Figure 1.

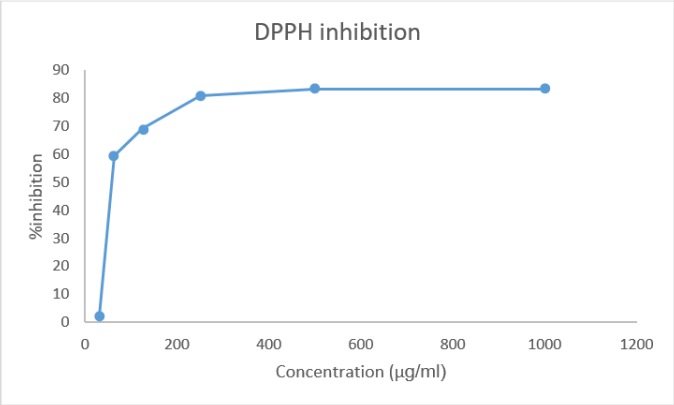

Figure 1 Scavenging of DPPH stable radical by volatile oil of *E. larica* from Oman.

Table 2 Antioxidant activity of volatile oil from *Euphorbia larica* collected in Oman

S. No	Concentration (µg/ml)	Percentages inhibition
1	31.25	2.38
2	62.5	59.52
3	125	69.04
4	250	80.95
5	500	83.33
6	1000	83.33

Antibacterial activity

Antibacterial activity of *E. larica* volatile oil against gram positive and gram-negative bacteria is shown in Table 3. The essential oil was tested at two doses 10 and 20 µL. Ampicillin was used as a positive control

Table 3 Antibacterial activity of volatile oil from *E. larica* collected from Oman

Microorganism	*E. larica* volatile oil		Ampicillin
	10µL	20µL	(20µg/ml)
E. coli (ATCC 25922)	9	11	30
P. vulgaris (ATCC 8472)	7	8	34
K. pneumoniae (ATCC 13883)	6	6	31
S. aureus (ATCC 25923)	8	10	34
S. agalactiae (ATCC 12386)	6	6	32

Cytotoxic activity of *Euphorbia Larica*

Table 4 shows percentage mortality of brine shrimp larvae exposed to various concentrations of volatile oil obtained from *Euphorbia larica.* The maximum mortality observed at the highest concentration was 50%.

LC_{50} value

The LC_{50} values of volatile oil of *Euphorbia larica* was deduced by regression analysis of the percent mortality values shown in Figure 2. From the calibration equation the volatile oil was found to have LC_{50} = 583.6576 µg/ml.

Table 4 Mean mortality of brine shrimp larvae when exposed to volatile oil from *Euphorbia larica* (n = 10 larvae per treatment)

S. No	Concentration (µg/ml)	Precentage mortality
1	31.25	0
2	62.5	0
3	125	10
4	250	40
5	500	50
6	1000	60

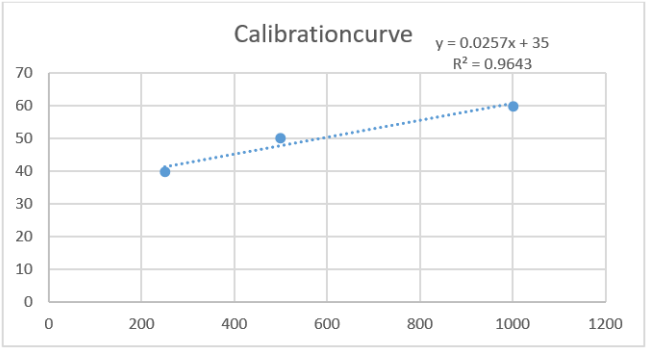

Figure 2 Regression analysis of the percent mortality values.

Discussion

GC-MS analysis was conducted and identified 22 compounds. The chemical composition is dominated by monoterpenes (63.6%) followed by sesquiterpenoids 24.16%. Triterpenoids accounted for 9.2% while diterpenes, content was 2.8%. According to the table, Terpinen-4-ol acetate, (*1R)*-α-Pinene and Eucalyptol are presented in highest percentage. Terpinen-4-ol acetate is 44.4% which is maximum as compared to other constituents followed by (*1R)*-α-Pinene (31.7%) and Eucalyptol (10.27%).

The major constituents are known to exhibit potential pharmacological activities. For instance, Terpinen-4-ol which is also a major constituent of the tea tree oil has many pharmacological activities including antimicrobial, antivirulent, antioxidant, anti-inflammatory, anti-hypertensive, and anti-cancer effects[11] (*1R)*-α-Pinene is a powerful antioxidant, anti-inflammatory, and anticarcinogenic agent.[12] Therefore, the observed pharmacological activities of *E. larica* essential oil could be associated to the presence of Terpinen-4-ol acetate (*1R)*-α-Pinene.

When working as antioxidants these molecules give up some of their electrons to neutralize free radicals. The sacrifice they make serves as a natural 'off" switch for the free radicals. This aids in stopping a chain reaction that may have an impact on other cell molecules. The results for the antioxidant activity presented in (Table 2) show that all concentrations show good activity starting from 31.5 µg/ml until 1000 µg/ml. The maximum inhibition was shown at 500 µg/ml. After increasing the concentration to 1000 µg/ml the antioxidant activity was not increased.

LC_{50} value of *Euphorbia larica* volatile oil was 583.6576 which showed that it has mild cytotoxic activity. In antibacterial test, at 10µL concentration all five types of gram-positive and gram-negative bacteria showed good inhibition. When the concentration is increased to 20µL then also the inhibition is increased, it means that *Euphorbia larica* volatile oil is showing dose dependent activity. It has a wide

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range of current and historical uses. In the past and the present. Phytochemicals and pharmacological investigations of *Euphorbia larica* showed that this plant has lots of medical potential and can be used in future as a medicine, therefore there is need of further pharmacological investigation on that plant.

Conclusion

GC-MS analysis detected Terpinen-4-ol acetate, (*1R*)-α-Pinene and Eucalyptol are the major compounds present in the volatile oil of *Euphorbia larica* whereas other similar studies which are conducted in different geographical region in Oman showed different results. This difference in result may be because of different weather and soil conditions. Northern Oman has humid weather because of surrounded by the ocean whereas Nizwa has a dry weather. The *Euphorbia larica* is grown in most regions of the Sultanate and is found in many areas in Oman. This plant is used by many Omanis, but they are not aware about the importance of this plant in terms of scientific value, so this project will spread awareness among people about the medicinal importance of that plant.

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Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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