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(RESEARCH ARTICLE)

Chemical composition and antimicrobial activity of essential oil of *Inula crithmoides* L. growing in Egypt

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Abstract

The study aimed to determine the chemical composition and antimicrobial properties of the essential oil of *Inula crithmoides* L. growing in Egypt. The essential oil was obtained from the flowers of *Inula crithmoides* L. with a yield of 0.18% v/w through hydro distillation. Gas chromatography-mass spectroscopy analysis was used to identify the chemical composition of the essential oil. The antimicrobial activity of the essential oil was tested against selected species of Gram-positive, Gram-negative bacteria, and *Candida* species using the disc diffusion method, and MICs were determined using the broth micro-dilution method. Twenty-two compounds were identified in the essential oil of *Inula crithmoides*, with the major components being thymol acetate (32.4%), p-cymene (15.3%), and thymol (14.3%). The essential oil exhibited significant antimicrobial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with an MIC of 25 µg/mL for *E. coli* and 50 µg/mL for other sensitive bacteria. The oil exhibited significant antifungal activity against all the tested *Candida* species with MIC 50 µg/mL. This study is the first to explore the chemical composition and antimicrobial activity of the essential oil of *Inula crithmoides* growing in Egypt, demonstrating its potential as a natural antiseptic agent.

Keywords: *Inula crithmoides*; Thymol acetate; *P*-Cymene; Antimicrobial; Essential oil

1. Introduction

Despite significant progress in medicine, infectious diseases caused by bacteria, fungi, viruses, and parasites continue to present significant health threats globally. This is especially true in developing nations where access to modern medications is limited, and instances of drug resistance are on the rise [1]. With the increase in drug resistance and the limited availability of specific antibiotics, there is an urgent need to investigate new antimicrobial agents primarily derived from natural sources to tackle these challenges [2]. Currently, research efforts focused on natural compounds and products primarily revolve around plants due to their local availability, cost-effectiveness, and potential for selection based on established ethnomedicinal claims for their applications [3].

Inula crithmoides L., golden samphire, is a perennial halophyte plant belonging to the genus *Inula* (Asteraceae family), growing up to 1m and flowers from July to August. It grows along the coasts of Europe, North Africa, and Western Asia in saline soils and can tolerate maritime exposure [4]. It is often used as cooked and raw food, especially in Lebanon, Spain, and Italy [5]. It could be considered a nutritionally interesting plant promoting the improvement of essential nutrients for human consumption, such as proteins, minerals, total dietary fiber, and low lipid content [6]. Previous phytochemical investigations on *I. crithmoides* showed the presence of triterpenoids, flavonoids, butyl glycosides, sesquiterpenes, quinic acid derivatives, diterpenes, thiophenes and several thymol derivatives [7-15]. Previous papers

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have been published on the composition of the essential oils of *I. crithmoides* collected from different places: Sicily, Central Italy, Tunisia, and Spain, Malta, and Greece [5, 16-19].

Several studies showed the antioxidant and antifungal properties of both the extracts and the essential oils of *I. crithmoides* [17, 18, 20-22]. Furthermore, the herbicidal [23], antileishmanial [24], and antibacterial [5, 12, 18, 25] properties of this species have also been determined. The genus *Inula* includes other species like *Inula viscosa* (L.) and *Inula cappa*, which have demonstrated effective antibacterial properties. For instance, the essential oil of *I. viscosa*, containing a significant amount of polygodial (19.8%), phytol (12.3%), fokienol (6.0%), and intermedeol neo (5.1%), exhibited moderate activity against *S. aureus* and *E. enterica* [26]. Similarly, the essential oil of *Inula cappa* displayed notable activity against *Enterococcus faecalis*, *Klebsiella pneumonia*, *Xanthomonas phaseoli*, and *Bacillus subtilis* [27]. No research papers were found investigating the essential oil of *Inula crithmoides* growing in Egypt. This research was planned to investigate the chemical composition and antimicrobial activity of the essential oil of *Inula crithmoides* L., which is growing in Egypt. The aim is to understand the specific chemical components present in this plant's essential oil and determine its effectiveness as an antimicrobial agent. This research can potentially contribute to developing natural antimicrobial products and pharmaceuticals.

2. Material and methods

2.1. Plant material

Flower heads of *Inula crithmoides* were collected from the North Coast, Egypt, at their flowering stage (July 2023). The plants were kindly previously identified by the late Professor Mohammed AlGebali, Professor of Plant Taxonomy, National Research Center, Dokki, Giza, Egypt. A voucher herbarium specimen had been deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

2.2. Preparation of the essential oil

The essential oil was prepared from the fresh flowers of *Inula crithmoides* (500 g) by hydro distillation using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate and stored in sealed glass vials at 4-6 ^{o}C before analysis. The percentage yield was determined according to European Pharmacopoeia 2004 [28].

2.3. GC/ MS analysis of the essential oil

The essential oil was analyzed using GC/MS with a Shimadzu GC/MS – QP 5050 A and an HP-5 MS capillary column. The analysis involved using helium as the carrier gas and an ionization mode of EL (70 ev). The temperature program started at 40 °C for 2 minutes, then increased gradually at 2°C per minute until reaching 250 °C, remaining for 8 minutes. The detector and injector temperatures were both set at 250 °C. The data was searched against the Wiley library using Software Class 5000.

2.4. Identification of the essential oil

The compounds were identified by comparing their retention indices (RI) on the HP-5 MS column to those of C5- C24 *n*-alkanes found in the literature. This was supplemented by a library search using the Willey database 229 LIB and comparing mass fragmentation patterns with available references and published data [5, 16-19, 29-32]. The percentage composition of the essential oil was determined using computerized peak area measurements. The results, calculated as mean values after three injections, are presented in Table 1.

2.5. Test organisms

The pure bacterial strains obtained from the American Type Culture Collection (ATCC) included Gram-positive strains such as *Staphylococcus aureus* ATCC 13709, *Staphylococcus epidermidis* ATCC 35984, *Streptococcus pyogenes* ATCC 19615, and *Bacillus subtilis* ATCC 6051. In addition, Gram-negative strains such as *Escherichia coli* ATCC 9637, *Klebsiella pneumoniae* ATCC 1705, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, and *Shigella boydii* ATCC 9905 were used, along with pure strains of *Candida albicans* ATCC 10231, *C. glabrata* ATCC 90030, *C. krusei* ATCC 14243, and *C. parapsilosis* ATCC 22019. These microorganisms were provided by the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

2.6. Testing for antimicrobial activities

Minimum Inhibitory Concentrations (MICs) were determined using the broth microdilution method [33,34]. Test yeasts or bacteria strains were suspended in media, and the cell densities were adjusted to 0.5 McFarland standards at 530 nm

wavelength using a spectrophotometric method. Inoculums (0.1 mL) were added to the microtiter plates, which were incubated in a humid atmosphere at 30 °C for 24–48 h (yeast) or at 37 °C for 24 h (bacteria). In addition, positive (medium with inoculums but with no essential oil) and negative (Uninoculated medium, 200 µL) growth controls were prepared. The growth in each well was compared with the growth in the control wells. MICs were visually determined and defined as the lowest concentration of the essential oil produced ≥ 50% growth inhibition for yeast and ≥95% growth reduction for bacteria compared with the growth in the control well. Each experiment was performed in triplicate, and the results were calculated as mean values and presented in Table 2.

2.7. Statistical analysis

The results were presented as the mean plus or minus the standard deviation. The experimental findings were analyzed using the variance analysis method. Variations were considered statistically significant at a P level of less than 0.05 [35].

3. Results

3.1. GC-MS analysis of essential oil

The yield of essential oil prepared by hydro distillation from the flowers of *I. crithmoides* was 0.18 % v/w. The results of GC-MS analysis of the essential oil are presented in Table 1, which reports the percentage composition of the essential oil; twenty-two compounds were detected and identified. The oil is mainly constituted by oxygenated monoterpenes (57.1%), monoterpene hydrocarbons constitute 38.7%; thymol acetate is the main constituent (32.4%) followed by *p*-cymene (15.3%). Sesquiterpene hydrocarbons constitute 2.8% of oil composition, and spathulenol (1.4%) is the only oxygenated sesquiterpene identified in the oil. This is the first study of the chemical composition of the essential oil of *I. crithmoides*, growing in Egypt.

Table 1 Chemical composition of the essential oil of *I. crithmoides*

No.	KI*	Compound	Formula	Percentage (Mean ± SD)**
1	932	*α*-Pinene	$C_{10}H_{16}$	3.7 ± 0.0
2	944	Camphene	$C_{10}H_{16}$	2.2 ± 0.0
3	978	*β*-Pinene	$C_{10}H_{16}$	1.4 ± 0.0
4	980	*β*-Myrcene	$C_{10}H_{16}$	6.7 ± 0.0
5	998	*α*-phellandrene	$C_{10}H_{16}$	1.2 ± 0.0
6	1013	*p*-Cymene	$C_{10}H_{16}$	15.3 ± 0.0
7	1022	*β*-phellandrene	$C_{10}H_{16}$	3.8 ± 0.0
8	1041	*o*-Cymene	$C_{10}H_{14}$	0.4 ± 0.0
9	1029	Limonene	$C_{10}H_{16}$	1.7 ± 0.0
10	1030	*β*-Phellandrene	$C_{10}H_{16}$	2.1 ± 0.0
11	1067	Thymol	$C_{10}H_{14}O$	14.3 ± 0.0
12	1086	Terpinolene	$C_{10}H_{16}$	0.2 ± 0.0
13	1167	Borneol	$C_{10}H_{18}O$	3.7 ± 0.0
14	1189	α-Terpineol	$C_{10}H_{18}O$	0.5 ± 0.0
15	1228	Isobornyl formate	$C_{11}H_{18}O_2$	6.2 ± 0.0
16	1352	Thymol acetate	$C_{12}H_{16}O_2$	32.4 ± 0.0
17	1377	α-Copaene	$C_{15}H_{24}$	0.6 ± 0.0
18	1390	β-Elemene	$C_{15}H_{24}$	0.2 ± 0.0
19	1407	Longifolene	$C_{15}H_{24}$	0.7 ± 0.0
20	1418	β-Caryophyllene	$C_{15}H_{24}$	0.9 ± 0.0
21	1424	β-Cedrene	$C_{15}H_{24}$	0.4 ± 0.0
22	1576	Spathulenol	$C_{15}H_{24}O$	1.4 ± 0.0

* Kovats retention index on HP-5 MS column ** n=3

Thymol acetate (32.4%) was the principal constituent, followed by *p*-cymene (15.3%). In comparison, 2,3,4-trimethylacetophenone (5.3%) [5], which was previously detected in the essential oil of *I. crithmoides* aerial part collected from Italy is absent in the essential oil isolated from the flowers of *I. crithmoides* growing in Egypt. 1-methylethyl-trimethylbenzene (18.7%), scopoletin (15.3%) which were previously detected in the essential oil of *I. crithmoides* aerial parts growing in Italy [17] were absent in the oil under investigation.

The essential oil obtained from plants collected in Italy [5, 16, 17], Tunisia [18], Spain, Malta, and Greece [19], was dominated by monoterpene hydrocarbons (32.1–87.4%). The oil under investigation is dominated by oxygenated monoterpenes (57.1%), followed by monoterpene hydrocarbons (38.7%). α-phellandrene (0.9–26.2%), *p*-cymene (trace-53.8%), β-phellandrene (6.6- 30.7%), limonene (3.2–24.0%), which are present in our sample in percentages 1.2, 15.3, 2.1, 1.7, respectively. On the other hand, β-myrcene (6.7%) was previously detected only in the oil prepared from the plant collected from Sicily, Italy [5]. Sesquiterpene compounds are absent in some essential oils [5, 17, 18] while present in others [16, 19]. Thymol acetate was also the predominant oil constituent in Sicilian oil (14.4%) [5]. Finally, the plant's essential oil under investigation is characterized by the occurrence of borneol, isobornyl formate, and spathulenol.

3.2. Evaluation of antimicrobial activity

The analysis of the antimicrobial activity, performed using the agar disc diffusion method and MIC measurements, demonstrated that the essential oil of *I. crithmoides* has significant antibacterial and antifungal properties (Table 2). It notably hindered the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*, with a MIC of 50 µg/mL. Moreover, the essential oil exhibited significant inhibitory effects on the growth of the Gram-negative bacteria, *E. coli* and *Pseudomonas aeruginosa*, with MIC values of 25 and 50 µg/mL, respectively. However, it displayed significant antifungal activity against all the tested species with MIC of 50 µg/mL.

Table 2 Antimicrobial activity and MIC of essential oil of *I. crithmoides*

Microorganism	**DD[a] (M± SD)**		**MIC[b] (µg/mL)**	
	Essential oil	**Standard**	**Essential oil**	**Standard**
Gram +ve Bacteria		**Gentamycin**		**Gentamycin**
Staphylococcus aureus	20.3 ± 0.4*	24. 6 ± 0.5	50	8×10^{-3}
Staphylococcus epidermidis	19.2 ± 0.3*	25.3 ± 0.4	50	1×10^{-2}
Streptococcus pyogens	10.3 ± 0.2	24.5 ± 0.3	NT	NT
Bacillus subtilis	17.2 ± 0.5 *	26.4 ± 0.4	50	1×10^{-2}
Gram -ve Bacteria		Gentamycin		Gentamycin
Escherichia coli	22.5 ± 0.4*	26.7 ± 0.9	25	8×10^{-3}
Klebsiella pneumonia	12.8 ± 0.2	23.7 ± 0.6	NT	NT
Proteus vulgaris	10.4 ± 0.1	25.5 ± 0.5	NT	NT
Pseudomonas aeruginosa	20.5 ± 0.2*	22.4 ± 0.3	50	1×10^{-2}
Shigella boydii	12.7 ± 0.2	21.8 ± 0.4	NT	NT
Fungi		Nystatin		Nystatin
Candida albicans	14.2 ± 0.2*	15.4 ± 0.1	50	1×10^{-2}
Candida glabrata	15.8 ± 0.2*	16.7 ± 0.4	50	1×10^{-2}
Candida krusei	13.6 ± 0.1*	15.6 ± 0.3	50	1×10^{-2}
Candida parapsilosis	13.0 ± 0.2*	15.8 ± 0.1	50	1×10^{-2}

DD[a], agar disc diffusion method. Diameter of the inhibition zone (mm) including the disc diameter of 6 mm; MIC[b], minimum inhibitory concentration; values are given as µg/mL; NT, not tested; M ± SD, mean ± standard deviation (n=3). *significant at $p < 0.05$

4. Discussion

The composition of the essential oil is greatly different from that previously reported. The difference in composition is most likely attributed to climate, soil composition, altitude, age, and species variation [36]. Thymol acetate (32.4%) was the principal constituent, followed by *p*-cymene (15.3%). In comparison, 2,3,4-trimethylacetophenone (5.3%) [5], which was previously detected in the essential oil of *I. crithmoides* aerial part collected from Italy is absent in the essential oil isolated from the flowers of *I. crithmoides* growing in Egypt. 1-methylethyl-trimethylbenzene (18.7%), scopoletin (15.3%) which were previously detected in the essential oil of *I. crithmoides* aerial parts growing in Italy [17] were absent in the oil under investigation.

The essential oil from plants collected in Italy, Tunisia, Spain, Malta, and Greece was mostly made up of monoterpene hydrocarbons [5, 16-18]. In contrast, the oil under investigation is dominated by oxygenated monoterpenes, it contains α-phellandrene, p-cymene, β-phellandrene, and limonene in percentages of 1.2, 15.3, 2.1, and 1.7, respectively. Additionally, it contains β-myrcene, previously only found in oil from plants collected in Sicily, Italy [5]. Some previously studied essential oils lack sesquiterpene compounds [5, 17, 18], while others contain them [16, 19]. Thymol acetate was the predominant oil constituent in the Sicilian oil, at 14.4% [5]. Lastly, the essential oil of the plant under investigation is characterized by borneol, isobornyl formate, and spathulenol.

Several researchers have previously defined the antimicrobial activity of the major compounds of the investigated oil. The oil components, namely thymol [37], *p*-cymene [38], β-myrcene [39], borneol [40], α-terpineol [41], α-pinene and β-pinene [42], have been reported to display antibacterial activity against several bacterial and fungal species. The presence of these compounds in the essential oil of *I. crithmoides* may account for its observed antimicrobial activity.

The results of this study on the chemical composition and antimicrobial activity of *I. crithmoides* essential oil are the first to be reported. These findings support the observations of some other researchers, who proved that the essential oil of *I. crithmoides* showed inhibitory activity against *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [18], *Tholu Bommalu* [5] growth but no activity against *Escherichia coli* [18].

5. Conclusion

This study investigated the chemical composition and antimicrobial activity of the essential oil of flowers of *I. crithmoides* growing in Egypt for the first time. The oil is rich in oxygenated monoterpene constituents, especially thymol acetate, followed by monoterpene hydrocarbons mediated by *p*-cymene and showed significant *in vitro* antibacterial and antifungal activity against tested microorganisms. Further, *in vivo* studies are needed to ensure the antibacterial activity and possible use of the essential oil of *I. crithmoides* as an antimicrobial agent.

Compliance with ethical standards

Disclosure of conflict of interest

The author declares no conflict of interest.

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