

CHEMICAL COMPOSITION, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OIL OF PULICARIA JAUBERTII FROM SOUTH YEMEN

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ABSTRACT

The chemical composition of the essential oil obtained from the flowers of *Pulicaria jaubertii* Gamal-Eldin (syn. *Pulicaria orientalis* Jaub.) was analyzed by GC-MS. The oil of *P. jaubertii* oil was strongly dominated by p-Menth-6-en-2-one (carvotanacetone) (93.5%) followed by chrysanthenone (0.9 %) and borneol (0.7 %). The antimicrobial activity of the essential oil was evaluated against five microorganisms, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*, using disc diffusion and broth microdilution methods. The oil showed the strongest bactericidal activity against Gram-positive bacteria *S. aureus* and was active against *C. albicans* as well. The free radical scavenging ability of the

oil was assessed by the DPPH assay to show antiradical activity with an IC₅₀ of 280 µg/mL.

KEYWORDS: Essential oil, *Pulicaria jaubertii*, carvotanacetone, GC-MS, Antimicrobial, Antioxidant.

INTRODUCTION

The genus *Pulicaria*, family Asteraceae (Compositae), is represented by ca. 100 species, more than 10 of which grow wild in Yemen.^[1] Moreover, about seven endemic species of *Pulicaria* grow wild in Soqatra Island, some of which have been used traditionally as antiseptic and/or food additives.^[2] Several studies investigated earlier the chemical compositions of the essential oils of numerous species of the *Pulicaria* genus collected from different world localities.^[3,4,5,6,7,8,9,10,11] Two investigated *Pulicaria* species were from North Yemen^[12,13] and two from South Yemen.^[14,15] The essential oil of *P. jaubertii* (EOPJ) from Saudi Arabia was investigated for its chemical composition and biological activities^[11] where EOPJ from North Yemen was investigated for only its chemical composition focused on the leaf parts.^[12] There have been no reports on the biological activities of Yemeni EOPJ. As part of our program to evaluate essential oils from Yemeni and Soqotri flora.^[14,15,16,17,18] this work reports for the first time the chemical composition, antimicrobial and antioxidant activity of the essential oil obtained from the flowers of *P. jaubertii* and collected from South Yemen. The plant is used as an aromatic cosmetic agent for women^[1] and known locally (Khooa).

MATERIALS AND METHODS

Plant material

The flowers of *P. jaubertii* were collected at flowering stages from Lahj province, South Yemen, in March, 2010. The plant was identified by Pharmacognosy Department, Faculty of Pharmacy, Aden University. A voucher specimen of the plant material (YEMP-ABY-10) has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Aden University.

Essential oil extraction

Dried flowers (30 g) from *P. jaubertii* were hydrodistilled for 3 h in a Clevenger type apparatus according to the European Pharmacopoeia.^[19] The obtained oil was subsequently dried over anhydrous Na₂SO₄ and kept at 4°C until analysis.

GC-MS analysis

Analytical GC-MS system consisting of an Agilent 6890N gas chromatograph and a mass selective detector (Agilent@5973 Network MSD) was used. Injection was done with Agilent@7683 Series Injector (Split 1:40 at 250°C, 2.0 µl; carrier gas: helium 1.1 mL/min (60 kPa) at 110°C; pressure rise: 6 kPa/min.). The MS operated in the electron impact mode with an ionization energy of 70 eV. The oven program started with 1min at 70°C, the oven temperature was increased at 3°C/min to 220°C. Full scan mass spectra were acquired from

35-350 m/z at a rate of 4.5 scans/s and with a 5.00 min solvent delay. Chromatography was performed using a 30 m DB-5 column (J&W Scientific, Folsom, USA) with 0.25 mm i.d. and 0.25 μm film thickness.

Determination of volatile compounds

The detected compounds were identified by processing of the raw GC-MS data with ChemStation G1701CA software and comparing with NIST mass spectral database 2.0 d (National Institute of Standards and Technology, Gaithersburg, USA) and from retention indices and mass spectra of standard compounds. Relative amounts of detected compounds were calculated based on the peak areas of the total ion chromatograms (TIC).

Antimicrobial Activity

Microorganisms

The following bacterial strains were employed in the screening: *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6059), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), as well as the fungal strain *Candida albicans* (ATCC 90028).

Disc diffusion assay

Modified agar diffusion method^[20] was used to determine antibacterial and antifungal activities. The bacterial cell suspension was prepared from a 24 h culture and adjusted to an inoculation of 1×10^6 colony forming units per ml. Sterile nutrient agar (Immunpräparate, Berlin, D, 26 g agar/L distilled water) was inoculated with bacterial cells (200 μL of bacterial cell suspension in 20 mL medium) and poured into dishes to give a solid medium. Yeasts and hyphomycetes (1×10^5 colony forming units per mL) were inoculated into sterile Mueller-Hinton-agar (Becton Dickinson, Heidelberg) according to DIN E 58940-3 for the agar disc-diffusion assay. The EO (10 μL for bacteria and 20 μL for the fungus) was applied on sterile paper discs (6 mm diameter, Schleicher and Schuell, D, ref. no. 321860). The discs were deposited on the surface of inoculated agar plates. Plates with bacteria were incubated for 24 h at 37 °C, plates with yeasts for 48 h at 36 °C. Inhibition zone diameters around each of the disc (diameter of inhibition zone plus diameter of the disc) were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated for the three replicates.

Determination of minimum inhibitory concentrations (MIC)

The broth micro-dilution method described by Mann and Markham^[21] with modifications was used to determine the MIC of investigated volatile oil against the above tested standard microbial strains. With sterile round-bottom 96-well plates, duplicate two-fold serial dilutions of EO (100 μ L/well) were prepared in the appropriate broth containing 5 % (v/v) DMSO. Two-fold dilutions of ampicillin, gentamycin, nystatin were used as a positive control. A bacterial cell suspension (prepared in the appropriate broth) of 100 μ L, corresponding to 1×10^6 CFU/mL, was added in all wells except those in columns 10, 11 and 12, which served as saline, EO and media sterility controls, respectively. Controls for bacterial growth without EO were also included on each plate. The final concentration of bacteria in the assay was 5×10^5 CFU/mL. Plates were then incubated at 37 °C for 18 h overnight. After incubation, the MIC of each EO was determined as the lowest concentration at which no growth was observed in the duplicate wells. Twenty microliters of a p-iodonitrotetrazolium violet solution (0.04 %, w/v) (Sigma, USA) was then added to each well. The plates were incubated for a further 30 min, and estimated visually for any change in color from yellow to pink indicating reduction of the dye due to bacterial growth. The highest dilution (lowest concentration) that remained yellow corresponded to the MIC. Experiments were performed in duplicate.

Determination of radical scavenging activity

The free radical scavenging activity was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. This assay measures the free radical scavenging capacity of the investigated essential oils. Qualitative determination was done as described in Sievers^[22] Quantitative estimation was carried out according to the method of Brand.^[23] The reaction mixture (total volume 1 mL) contained 500 μ L of test essential oil and 125 μ L of DPPH in ethanol. Different concentrations of test volatile oil (10, 50, 100, 500 and 1000 μ g/mL) were prepared while the concentration of DPPH was 1 mM in the reaction mixture. These reaction mixtures were taken into Eppendorf tubes and incubated at 37 °C for 30 min, the absorbance was measured at 517 nm. Percent radical scavenging activity by sample treatment was determined by comparison with ethanol treated control group. Ascorbic acid was used as positive control. The DPPH radical concentration was calculated using the following equation: Radical scavenging activity (%) = $\frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$

Table1. Chemical composition of essential oil of *P. jaubertii* flowers

RI	Compounds ^a	Content %
985	β -Pinene	0.3
1075	cis-Linalool oxide	0.3
1077	Linalool	0.4
1098	Chrysanthenone	0.9
1124	trans-p-2,8-Menthadien-1-ol	0.3
1149	Borneol	0.7
1166	Phellandrene-8 α -ol	0.3
1173	Terpinen-4-ol	0.2
1188	α -Terpineol	0.3
1209	Caprylyl acetate	0.2
1227	Nerol	0.1
1237	p-Menth-6-en-2-one = (+)-Carvotanacetone	93.5
1258	Geraniol	0.3
1273	p-(1-Propenyl)anisole	0.6
1410	Methyl eugenol	0.4
1487	β -Selinene	0.2
1586	Caryophyllene oxide	0.1
	Total identified	99.1

^aCompounds listed in order to their elution on the DB-5 column

RI: Retention indices on the DB-5 column relative to C10-C20 n-alkanes

RESULTS AND DISCUSSIONS

Hydrodistillation of the dried flowers of *P. jaubertii* gave yellow colored oil with a perfumery odor and yielded 0.84% on a dry weight basis. The chemical composition of EOPJ flowers analyzed by GC-MS is presented in Table 1, where compounds are listed in order of their elution from a DB5 column. Seventeen components were identified representing 99.1% of the total oil content, with the main compounds carvotanacetone (93.5%) followed by chrysanthenone (0.9 %) and borneol (0.7 %). Carvotanacetone has been reported in *Pulicaria* species.^[3,15] Although carvotanacetone has been found in other *Pulicaria* species EOs such *Pulicaria inuloides* leaves collected from North Yemen (47.3%).^[13] *Pulicaria undulata* leaves collected from South Yemen (91.4%).^[14] *Pulicaria jaubertii* collected from North Yemen as leaves showed less amount of carvotanacetone (64.0%).^[12] while our result showed that *P. jaubertii* floral EO contains the highest amount of carvotanacetone (93.5%) among the Yemeni *Pulicaria* species. The essential oil of *Pulicaria jaubertii* leaves from southern Saudi Arabia had the highest proportion of carvotanacetone (98.6%).^[11] The diameters of the zones of inhibition and minimum inhibitory concentrations (MIC) of EOPJ for the microorganisms

tested are shown in Table 2. The oil showed good antibacterial activity against Gram-positive bacteria. *S. aureus* was the most sensitive microorganism tested, with the strongest inhibition zone (IZ) (30 mm) and lowest MIC (250 µg/mL) followed by *B. subtilis* (IZ 20 mm and MIC 500 µg/mL). It showed also good antifungal activity against *C. albicans* (IZ 25 mm and MIC 300 µg/mL). *P. aeruginosa* was the most resistant strain. Generally, the oxygenated monoterpenes show more antimicrobial activity than hydrocarbon monoterpenes in the Eos.^[24] this explains the good antimicrobial activity of the EOPJ that is rich in oxygenated monoterpenes. Previous study reported that carvotanacetone showed moderate antimicrobial activity especially against *C. albicans*.^[25] Therefore the antimicrobial effect of EOPJ might be related to the presence of high content of carvotanacetone. On other hand, volatile terpene alcohols, such as borneol and chrysanthenone were reported to exhibited synergic antimicrobial effects.^[26] Therefore, the monoterpene alcohols that are indicated in the oil can support synergistic antimicrobial effects. In addition, the EOPJ showed moderate antioxidant activity (IC₅₀ = 280 µg/mL) compared with ascorbic acid (IC₅₀ = 6 µg/mL). It was reported that some EOs oxygenated monoterpenes such carvotanacetone^[14] and borneol^[27] exhibit antioxidant potentials.

Table 2: Antimicrobial activity of *P. jaubertii* flower essential oil

Test microorganisms	Essential oil		Standard Antibiotics Inhibition zone ^a (mm)		
	Inhibition zone ^a (mm) 10 µL/disc	MIC µg/mL	Ampicillin 10 µL/disc	Gentamycin 10 µL/disc	Nystatin 100 µL/disc
<i>Staphylococcus aureus</i>	30	250	17	25	n.t.
<i>Bacillus subtilis</i>	20	500	n.t.	26	n.t.
<i>Escherichia coli</i>	15	1000	28	23	n.t.
<i>Pseudomonas aeruginosa</i>	resistance	n.t.	29	28	n.t.
<i>Candida albicans</i>	25	300	n.t.	n.t.	23

^aInhibition zone diameter (mm), including 6-mm-diameter sterile disc, n.t. = not tested

CONCLUSION

From our results, it could be concluded that EOPJ is characterized by the high content of oxygenated monoterpenes (98.6%) mainly (+)-carvotanacetone with extreme high content (93.5%) that seem to have important chemotaxonomic value to differentiate between *P. jaubertii* growing in South and North Yemen. Moreover EOPJ showed good antimicrobial activity against human pathogenic microorganisms and considerable antiradical activity that may explain its traditional use as an antiseptic and cosmetic agent in South Yemen.

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