WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 01-09. Research Article ISSN 2277– 7105

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

Mohamed Al-Fatimi[1], Nasser A. Awadh Ali[*2], Martina Wurster[3], Saeed S. Al-Sokari[4] Ulrike Lindequist[3], William N. Setzer[5]

[1]Faculty of Pharmacy, Department of Pharmacognosy, Aden University,P.O.Box 5411 (Maalla). Aden. Yemen.

[2]Pharmacognosy Department, Faculty of Clinical Pharmacy Albaha University, KSA.

[3]Institute of Pharmacy, Department of Pharmaceutical Biology, Ernst-Moritz-Arndt-University Greifswald, D-17487 Greifswald, Germany.

[4]Department of Biology, Faculty of Sciences, Albaha University, KSA.

[5]Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.

Article Received on
18 Sept 2015,

Revised on 10 Oct 2015,
Accepted on 1 Nov 2015,

***Correspondence for Author**
Mohamed Al-Fatimi
Faculty of Pharmacy, Department of Pharmacognosy, Aden University, P.O.Box 5411 (Maalla). Aden. Yemen.

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 IC_{50} 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 Soqotra 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 Na_2SO_4 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 1×106 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 µL/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 µL, 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 µL of test essential oil and 125 µL of DPPH in ethanol. Different concentrations of test volatile oil (10, 50, 100, 500 and 1000 µg/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 (%) = Absorbance control – Absorbance sample/Absorbance control × 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-8α-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

[a]Compounds 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 (IC_{50} = 280 µg/mL) compared with ascorbic acid (IC_{50} = 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
Staphylococcus aureus	30	250	17	25	n.t.
Bacillus subtilis	20	500	n.t.	26	n.t.
Escherichia coli	15	1000	28	23	n.t.
Pseudomonas aeruginosa	resistance	n.t.	29	28	n.t.
Candida albicans	25	300	n.t.	n.t.	23

[a]Inhibition 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.

REFRENCES

1. Wood JRI. A Handbook of the Yemen Flora, Whitstable Litho Printers Ltd, Royal Botanic Gardens, Kew. UK, 1997; 296.
2. Miller GA, Morris M. Ethnoflora of the Soqotra Archipelago. Charlesworth Group, Huddersfield, UK, 2004; 8.
3. Ali AA, Makboul MA, Assaf MH, Anton R. Essential oil of Pulicaria undulata L. growing in Egypt and its effect on animal behavior. Bulletin of Pharmaceutical Sciences, Assiut University, 1987; 10: 37-49.
4. EL-Kamali HH, Yousif MO, Ahmed OI, Sabir SS. Phytochemical analysis of the essential oil from aerial parts of Pulicaria undulata (L.) Kostel from Sudan. Ethnobotanical Leaflets, 2009; 13: 467-471.
5. Nematollahi F, Rustaiyan A, Larijani K, Nadimi KM, Masoudi S. Essential oil composition of Artemisia biennis Willd. and Pulicaria undulata (L.) C.A. Mey., two Compositae herbs growing wild in Iran. Journal of Essential Oil Research, 2006; 18: 339-341.
6. Znini M, Cristofari G, Majidi L, Paolini J, Desjobert JM, Costa J. Essential oil composition and antifungal activity of Pulicaria mauritanica Coss., against postharvest phytopathogenic fungi in apples. LWT - Food Science and Technology, 2013; 54(2): 564–569.
7. El-Kamali HH, Ahmed AH, Mohammed AS, Yahia, AAM, El- Tayeb IH, Ali AA. Antibacterial properties of essential oils from Nigella sativa seeds, Cymbopogon citratus leaves and Pulicaria undulata aerial parts. Fitoterapia, 1998; 69: 77–78.
8. Liu LL, Yang JL, Shi YP. Phytochemicals and biological activities of Pulicaria species. Chemistry & Biodiversity, 2010; 7: 327-349.
9. Karim EI, Ishag KE, Elegami AAB, Mahmoud EN, Abu-AL-Futuh I. D-Carvotanacetone from Pulicaria undulata. Fitoterapia, 1992; 63: 281.
10. Mossa JS, Hifnawy MS, Al-Yahya MA, Al-Meshal IA, Mekkawi AG. Aromatic plants of Saudi Arabia – GC/MS analysis of essential oils of Pulicaria arabica and P. undulata. Pharmaceutical Biology, 1987; 25: 113-119.
11. Fawzy GA, Al Ati HY, El Gamal AA. Chemical composition and biological evaluation of essential oils of Pulicaria jaubertii. Pharmacognosy Magazine, 2013; 9: 28–32.
12. Algabr MN, Ameddah S, Menad A, Mekkiou R, Chalchat JC, Benayache S, Benayache F. Essential oil composition of Pulicaria jaubertii from Yemen. International Journal of Medicinal and Aromatic Plants, 2012; 2: 688–690.

13. Al-Hajj NQM, Wang HX, Ma C, Lou Z, Bashari M, Thabit R. Antimicrobial and antioxidant activities of the essential oils of some aromatic medicinal plants (Pulicaria inuloides– Asteraceae and Ocimum forskolei–Lamiaceae. Tropical Journal of Pharmaceutical Research, 2014; 13: 1287–1293.
14. Ali NAA, Sharopov FS, Alhaj M, Hill GM, Porzel A, Arnold N, Setzer WN, Schmidt J, Wessjohann L. Chemical composition and biological activity of essential oil from Pulicaria undulata from Yemen. Natural Product Communications, 2012; 7: 257–260.
15. Ali NAA, Crouch RA, Al-Fatimi MA, Arnold N, Teichert A, Setzer WN, Wessjohann L. Chemical composition, antimicrobial, antiradical and anticholinesterase activity of the essential oil of Pulicaria stephanocarpa from Soqotra. Natural Product Communications, 2012; 7: 113–116.
16. Al-Fatimi M, Wurster M, Schröder G, Lindequist U. In vitro antimicrobial, cytotoxic and radical scavenging activities and chemical constituents of the endemic Thymus laevigatus (Vahl). Records of Natural Products, 2010; 4: 49–63.
17. Ali NAA, Al-Fatimi, M.A, Crouch RA, Denkert A, Setzer WN. Antimicrobial, antioxidant, and cytotoxic activities of the essential oil of Tarchonanthus camphoratus. Natural Product Communications, 2013; 8: 683–686.
18. Ali NAA, Wurster M, Arnold N, Teichert A, Schmidt J, Lindequist U, Wessjohann L. Chemical composition and biological activities of essential oils from the oleogum resins of three endemic Soqotraen Boswellia species, Records of Natural Products, 2008; 2: 6–12.
19. Council of Europe. European Pharmacopoeia, 3rd Ed., Strasbourg, Council of Europe Press, 1997; 121-122.
20. Bauer AW, Kirby WMM, Sheriss JC, Turck M. Antibiotic susceptibility testing by standardised single method. American Journal of Clinical Pathology, 1966; 45: 493–496.
21. Mann CM, Markham JL. A new method for determining the minimum inhibitory concentration of essential oils. Journal of Applied Microbiology, 1998; 84: 538-544.
22. Sievers A, Oshinowo L, Schultze W, Koch A, Richter R. Einfache duennschicht-chromatographische Pruefung auf antioxidative Verbindungen mit dem DPPH-test. C35. Camag Bibliography Service, 2000; 88: 14–15.
23. Brand WW, Cuvelier HE, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft & Technologie, 1995; 82: 25–30.

24. Knobloch E, Pauli A, Iberl B, Wies N, Weigand H. Mode of action of essential oil components on whole cells of bacteria and fungi in plate tests. In Bioflavour 87. Schreier P (Ed). Walter de Gruyther, Berlin, 1988; 287-299.
25. İşcan G, Kirimer N, Demirci F, Demirci B, Noma Y, Başer KH. Biotransformation of (-)-(R)-α-phellandrene: antimicrobial activity of its major metabolite. Chemistry & Biodiversity, 2012; 9(8): 1525-32.
26. Griffin SG, Wyllie G, Markham JL, Leach DN. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour and Fragrance Journal, 1999; 14: 322–332.
27. Bozin B, Mimica-Dukic N, Bogavac M, Suvajdzic L, Simin N, Samojlik I, Couladis M. Chemical composition, antioxidant and antibacterial properties of Achillea collina Becker ex Heimerl s.l. and A. pannonica Scheele essential oils. Molecules, 2008; 13: 2058-2068