Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(5):224-228
(http://derpharmachemica.com/archive.html)

Antibacterial and Antifungal Activities of the essential oil of *Pulicaria jaubertii* leaves

[1,3]Methaq Algabr*, [2]Nabil Al-Hajj, [3]Ameerh Jaber, [3]Amtellah Alshotobi, [3]Shaima'a Al-suryhi, [3]Gadah Whaban and [3]Nawal Alshehari

[1]Department of Chemistry, Laboratory of Chemistry, Faculty of Applied Sciences, University of Hajjah, P.O. Box 80004, Hajjah, Yemen
[2]Department of Chemistry, Marine Science and Biological Research Authority, Aden, P.O. Box 1231, Aden, Yemen
[3]Department of Chemistry, Laboratory of Chemistry, Faculty of Education, University of Hajjah, Yemen

---

ABSTRACT

Steam distillation of the essential oil of P. jauberti was performed using a Clevenger apparatus. Essential oils were analyzed by gas Gas Chromatography-flame ioziation detector (GC-FID) and Gas Chromatography coupled to chromatography–mass spectrometry (GC-MS). The majorchemical components identified in P. jaubertii essential oil include carvotanacetone (63.975%), 1-methyl-1,2-propanedione (5.887%), 2,5-dimethoxy-para-cymene (3.303%) and ar-curcumene (3.276%). The antimicrobial activity of the essential oil of P. jaubertii was evaluated against all tested microorganisms. P. jaubertii essential oil inhibited all tested microorganisms except Escherichia coli with a minimum inhibitory concentration (MIC) of 5.0 µg/mL against Staphylococcus aureus.

Keywords: *Pulicaria jaubertii*, Essential oil, Antimicrobial, Carvotancetone

---

INTRODUCTION

The flora of Yemen, where is very rich and heterogeneous. Species diversity is a result of considerable climatic variation, which enabled different species to survive in the different ecological habitats. Over 3000 plant species are possibly found in the mainland, and about 10% of them are endemic, comprised 467 plant species belonging to 244 genera from 71 Families[1].The genus *Pulicaria* belonging to the family Asteraceae (tribe Inuleae), consists of more than 77 species found throughout Europe , North Africa and Asia and five species of this genus reported from Yemen [2,3]. Members of this genus contain various bioactive compounds such as monoterpenes, flavonoids, acetylenes, isocomene, and sesquiterpene lactones [4]. Biological actions reported for Pulicaria species include the antibacterial and antispasmodic activities of *P. undulate*, *P. odora*, and *P. dysenterica*[5]. In addition, members of this genus have been traditionally used to repel insects, to reduce influenza and common cold symptoms, and to treat back pain, intestinal disorders, and inflammation [5].The *Pulicariacrisps* indigenous to Yemen, locally known as Anssif, is traditionally used as diuretic, pyritic conditions in urogenetic organs, and to cure fever. The flowers of *Pulicariainuloides* was also used as spice and to make various delicious foods. Some investigation reported that this species reveal antimicrobial, antifungal, antimalaria and insecticides properties [6].

The aim of this study was to investigate the antibacterial and antifungal activities of the leaves of *Pulicaria jaubertii* essential oil.

MATERIALS AND METHODS

Plant collection and identification

The aerial part of *Pulicaria jaubertii* (leaves) were collected in November 2015 during the flowering stage in the Aljararea (Hajjah - Yemen). The plant was identified by Pr. Abdellah Amine (Sana'a University). A voucher specimen of the plant material has been deposited at the department of biology (Sana'a University).

Extraction of essential oil

The fresh of aerial part of *Pulicaria jaubertii* (65g) were ground in a blender, and essential oils were obtained by hydrodistillation at 100 °C using a Clevenger-type apparatus for 5 h with 1 L distilled water. The extracted oils were dried over sodium sulfate (Na_2SO_4) and stored at 4 °C until use.

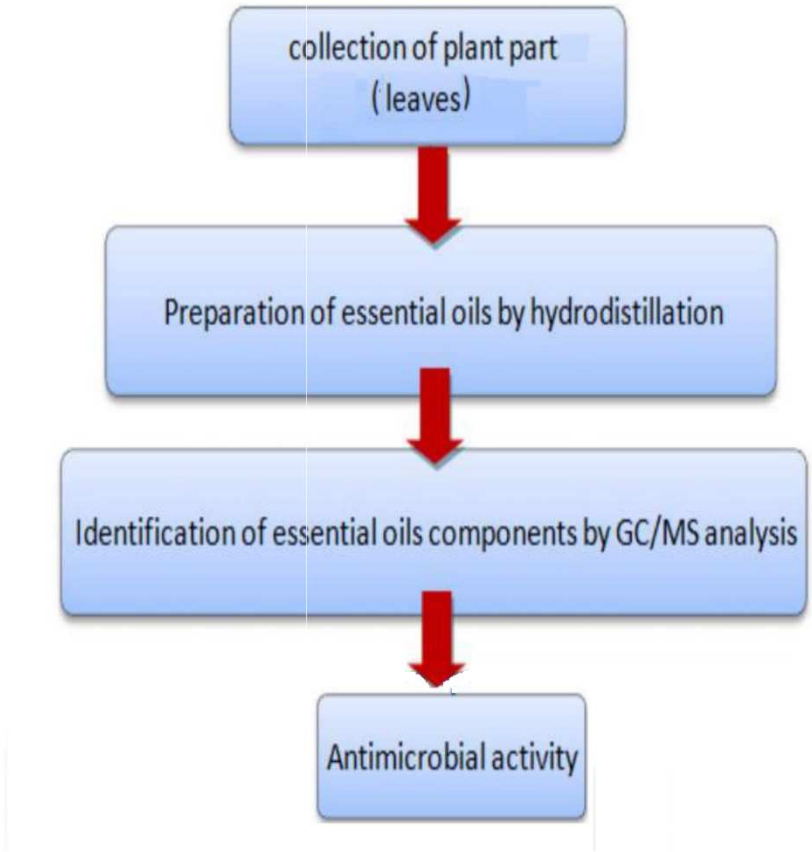

Figure 1. Experiment flowchart for isolation of essential oil

GC/MS analysis

The essential oil of *P. jaubertii* was analyzed on an Agilent gas chromatograph (GC- FID) Model 6890, equipped with a HP-5 MS fused silica capillary column having (5%- Phenyl)-methylpolysiloxane stationary phase (30 m length x 0.25 mm internal diameter and 0.25 µm film thickness), programmed from 50°C (5 min) to 250°C at 5°C/min and held for 5 min. Injector and flame ionisation detector temperatures were 280 and 300°C, respectively. The essential oil was diluted in acetone in 3.5% (v/v), and 1 µL was injected in split mode (1/60). Hydrogen was used as a carrier gas (1.0 mL/min). Solution of standard alkanes (C8-C26) was analyzed under the same conditions to cal- culate retention indices (RI) with Van Den Dool and Kratzequati [7].

ASSESSMENT OF ANTIMICROBIAL ACTIVITY

Microorganisms

Microorganisms were obtained from the Department of Microbiology, national general center laboratory, Sana'a .two strains of gram negative bacteria, Escherichia coli (ATCC 25922) *Pseudomonas aeruginosa* (ATCC 27853) and one strains of gram-positive bacteria *Staphylococcus aureus* (ATCC 29213) were used.as well as the one fungal strain *Candida albicans* (ATCC 90028). The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Disc diffusion assay

Antimicrobial activity of the essential oil was determined by using the disc diffusion assay [3] with some modifications. Muller Hintonagar was inoculated with the microorganism (10^4 colony forming units/mL). A 6-mm paper filter disc impregnated with 20 µL essential oil diluted in dimethyl sulfoxide was placed on the agar, and the oil was allowed to diffuse into the medium for 30 min at room temperature. The plates were then incubated at 37 °C for 20 h (bacteria) or at 33 °C for 72 h (yeast). The zone of inhibition was recorded as the mean ± standard deviation (SD) of triplicate experiments. Ampicillin (10 µg) and Ceftriaxone (10 µg) were used as reference antibiotics for bacteria, and amphotericin B (100 µg) was used as the reference antifungal agent for C. albicans.

Determination of minimal inhibitory concentration (MIC)

The MIC of the essential oil was determined using the microtiter broth microdilution assay described by Amsterdam [8]. The essential oil were diluted to 50 µL/mL and subjected to a serial dilution in a microtiter plate containing tryptic soy broth (for bacteria) or Sabouraud dextrose broth (for yeast). The bacterial and yeast strains were suspended in the liquid culture medium at a final concentration of 104colonyforming units/mL. After incubation at 37 °C for 24 h (bacteria) or at 32 °C for 72 h (yeast), optical density was measured at 520 nm using a spectrophotometer. MIC was defined as the lowest concentration of the essential oil at which the microorganisms did not exhibit visible growth.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

Ten components were identified in the essential oil of *P. jaubertii*, with Carvotanacetone (63.975%), 1-methyl-1,2-propanedione (5.887%), 2,5-dimethoxy-para-cymene (3.303%) and ar-curcumene (3.276%)being the major constituents (Table 1 and Figure 2).The yield of volatile oil of *P.jaubertii* obtained by steam distillation of the finely powdered leaves were 0.9 %. Essential oil extracted from *P.jaubertii* was light yellow in colour and with a perfumery odor.To the best of our knowledge, there is no any report on the chemical composition of *P. jaubertii* essential oil in the literature. There are few reports on the chemical composition of the oils from the other plants belonging to the genu of *P. jaubertii* previous studied on the composition of *P. dysenterica* and *O. basilicum* oils show that there are some qualitative and quantitative differences which, can be attributed to growth conditions, genetic factors, geographical variations and analytical procedures [5].The plant is important source of potential compounds for the development of new therapeutic agents. Plants phenolics are widely distributed in the tissues of plants as well as play a vital role in the highly effective free radical scavengers and antioxidant activity. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [9].Carvotanacetone has been found in *Pulicariainuloides* and *Pulicariaundulata* leaves collected from North Yemen (47.3% and 91.4% respectively) [3, 10].These compounds possess diverse biological activities, such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic activities [11].Differences in values may be due to differences in geographical areas, genus, reproductive stage, climate, temperature, humidity, season of harvest, and extraction methods [12].

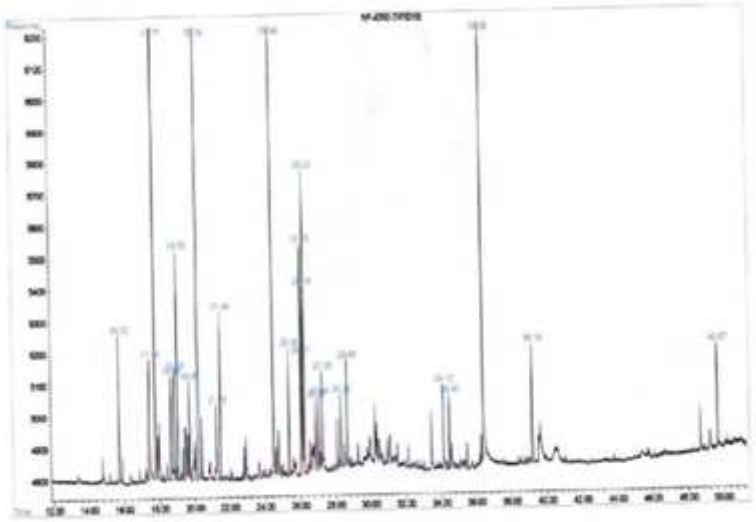

Fig 2: Chromatogram of *Pulicaria jaubertii* essential oil compounds

Table 1: Main components of the essential oil of *Pulicaria jaubertii*

Compound	RI	Content %
Linalool	1097	0.989
Benzoic acid	1154	1.674
1-methyl-1,2-propanedione	1165	5.887
α-Terpineol	1195	0.928
Menthan-2-one	1201	1.069
Unknown	1208	1.639
Thymol methyl ether	1232	0.691
Carvotanacetone	1250	63.957
Thymol	1287	0.740
Carvacrol	1296	1.161
2,5-dimethoxy-para-cymene	1410	3.303
Geranylacetone	1445	0.855
Unknown	1472	0.730
Unknown	1474	1.706
(E)-β-Ionone	1477	1.342
Ar-curcumene	1481	3.276
β-Bisabolene	1507	0.649
β-Sesquiphellandrene	1514	0.525
Δ-cadinene+unknown	1523	0.671
2-methyl-butyrate neryl	1568	0.524
caryophyllene oxide	1583	0.852
6,10,14-trimethyl-pentadecan-2-one	1838	0.534
Phthalate	1854	0.578
Hexadecanoic acid	1954	3.996
Octadecan-9-enoic acid	2104	0.910
unknown		0.813
Total		**99.999**

RI: Retention Index

Antimicrobial activity

The in vitro bacteriostatic activity of *P. jaubretii* essential oil was greater as assessed by the disc diffusion assay (Table 2 and Figure 3) and by the microtiter broth microdilution assay (Table 2 and Figure 3).In this study, *P.jaubertii* essential oils demonstrated higher antibacterial activities against all bacteria tested except *E. coli*. Furthermore, the Gram-positive bacteria *Staphylococcus aureus* was more sensitive to this essential oil than the Gram-negative bacteria *Escherichia coli*. Similarly, a previous study reported that the essential oils of *Pulicaria astephanocarpa* showed high antimicrobial activity against Candida albicans and all tested bacteria except *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Salmonella typhimurium* [13]. Our results suggest that *P. jaubertii* essential oils may be useful in the treatment of infectious diseases caused by *S. aureus* and *C. albicans*. This activity may be related to its high level of Carvotanacetone (63.975%), 1-methyl-1,2-propanedione (5.887%), 2,5-dimethoxy-para-cymene (3.303%) and ar-curcumene (3.276%).In addition, the presence of one phenolic isomers of thymyl acetate may synergistically contribute to the antifungal activity of Pulicaria essential oils. Phenolic compounds in essential oils (carvacrol, thymol, Hexadecanoic acid and Benzoic acid) are thought to be primarily responsible for their biological properties [14].

Minimal inhibitory concentration (MIC) values indicate that *P. jaubertii* essential oils have a greater inhibitory action against Gram-positive bacteria than Gram-negative bacteria (Table 2 and Figure 3). A previous study showed that the antimicrobial effects of spices and herbs against *C. albicans* and *E. coli*, and other Gram-negative bacteria were due to their complex chemical composition, which included compounds such as thymol, carvacrol, methyl eugenol, linalool, α-pinene, 1, 8-cineole, and camphor [15]. This discrepancy in antibacterial potential may be caused by variations in chemical composition, which may be influenced by the distillation and extraction techniques as well as geographical origin.

Table 2.Antimicrobial activity of *Pulicaria jaubertii* essential oil

Test microorganism	*P. juabertii* (zone of inhibition, mm) 5µg/disc	*P. juabertii* (zone of inhibition, mm) 10µg/disc	MIC	Standard antibioticb (zone of inhibition, mm)
Gram-positive bacteria				**Ampicillin**
Staphylococcus aureus	32.0	34.0	5.0	16mm
Gram-negative bacteria				**Ceftriaxone**
Pseudomonas aeruginosa	9.0	13.0	NT	6 mm
Escherichia coli	NT	NT	NT	NT
Yeast				**Amphotericin B**
Candida albicans	mm>	mm>	NT	mm>

[a]: includes diameter of disc(6 mm); NT: essential oil has no antimicrobial activity against this microorganism

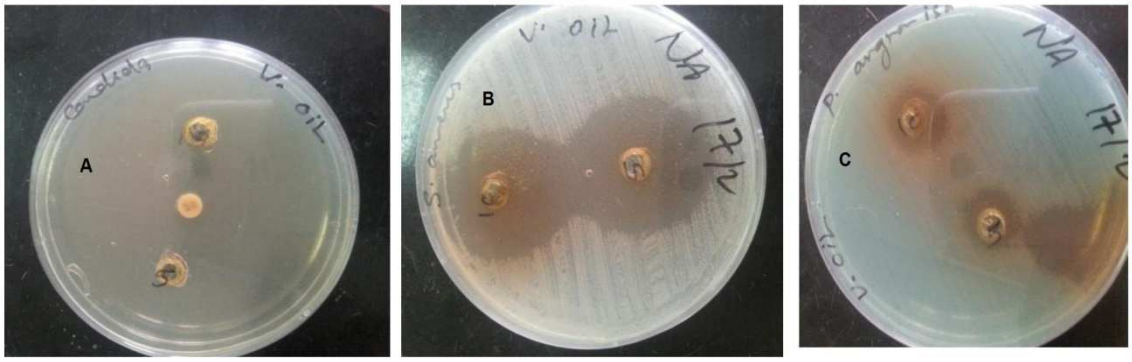

Fig (3). Inhibition zone of essential oil against (*Candida albicans, Pseudomonas aeruginosa, staphylococcus aureus*)
A*: inhibition zone of essential oil against Candida albicans complete inhibition for both*
B*: Inhibition zone ofessential oil against staphylococcus aureus in the right 10*µl*/ml and 5*µl*/ml*
C*: Inhibition zone of essential oil against Pseudomonas aeruginosa in the right 10*µl*/ml and 5* µl*/ml*

CONCLUSION

The essential oils of *P. jaubertii* exerted strong antimicrobial actions against gram-positive bacteria, *Pseudomonas aeruginosa* and C. albicans, whereas *E. coli* was not. The essential oil of *P. jaubertii* collected at Hajjah province in Yemen is mainly characterized by the presence of oxygenated monoterpenes especially carvotanacetone. Results showed minor differences with literature data. These differences might be due to growth conditions, genetic factors, geographical variations and analytical procedures.

Acknowledgement
The authors express their thanks to the students of Chemistry, Laboratory of Chemistry, Faculty of Education, University of Hajjah, Yemen for their technical assistance. Special thanks to the national general center laboratory in Sana'a for their cooperation.

REFERENCES

[1] N. Q. M.Al-Hajj,H. Rashid,H.xin Wang,R.Thabit, &M. M. Rashed, *American Research Thoughts* , **2014**, 1,2, 973-1000.
[2] A. S.Dubaie, &A. A. El-Khulaidi,.Medicinal and aromatic plants in Yemen, deployment-components of effective-uses. *Ebadi Center for studies and Publishing Sana'a-Yemen*, **2005**, 127.
[3] N. Q. M.Al-Hajj, H. X.Wang, C.Ma, Z.Lou, M.Bashari, &R.Thabit, *Tropical Journal of Pharmaceutical Research*, **2014,***13*(8), 1287-1293.
[4] F. E.Hanbali, M.Akssira, A.Ezoubeiri, F.Mellouki, A.Benherraf, A. M. Blazquez, &H.Boira, *Journal of ethnopharmacology*,**2005,***99,* (3), 399-401.
[5] H. H.El-Kamali, &S. A. T. Mahjoub, *Ethnobotanical Leaflets*,**2009,**(6), 6.
[6] M.Stavri, K. T.Mathew, A.Gordon, S. D.Shnyder, R. A.Falconer, &S.Gibbons, *Phytochemistry*, **2008,***69*,(9), 1915-1918.
[7] K. A.Kumar, S.Sharvanee, J.Patel, &R. K. Choudhary, *International Journal of Phytomedicine*, **2010,***2*,(4).
[8] D.Amsterdam, *Antibiotics in laboratory medicine*,**1996,** *4*, 61-143.
[9] T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori and Y. Fujita. *Chemical and Pharmaceutical Bulletin* **1989,** 37(,8), 2016-2021.
[10] N. A.Ali, F. S.Sharopov, M.Alhaj, G. M.Hill, A.Porzel, N.Arnold, &L. Wessjohann, *Natural product communications*,**2012,** *7*,(2), 257-260.
[11] S. M.Khamsah, G.Akowah, &I. Zhari, *Journal of Sustainability Science and Management*,**2006,** *1*,(2), 14-20.
[12] J.Bruneton, Pharmacognosie. Phytochimie des plantesmédicinales.2eme édition.Techniqueet Documentation Lavoisier. Paris.**1999,** p 915.
[13] D.Amsterdam, *Antibiotics in laboratory medicine*, **1996,***4*, 61-143.
[14] A.Ultee, M. H. J.Bennik, &R.Moezelaar, *Applied and environmental microbiology*,**2002,** *68*,(4), 1561-1568.
[15] S.Nanasombat, &P.Lohasupthawee, *Sci Tech J*, **2005,** *5*(3), 527-538.