Chemical composition and biological evaluation of essential oils of *Pulicaria jaubertii*

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ABSTRACT

Background: The present study reports and compares the results of Gas Chromatographic-Mass analyses of *Pulicaria jaubertii* leaf **(P-1)** and root **(P-2)** essential oils, as well as their *in vitro* antimicrobial and cytotoxic activities. **Materials and Methods:** The chemical composition of **P-1** and **P-2** essential oils of *P. jaubertii*, was investigated by GC-MS. Moreover, the essential oils were evaluated for their antimicrobial activity using the broth micro-dilution assay for minimum inhibitory concentrations (MIC). The crystal violet staining method (CVS) was used for evaluation of their cytotoxic activity on HEPG-2 and MCF-7 human cell lines. **Results:** This investigation led to the identification of 16 constituents in **P-1**, and 23 constituents in **P-2**, representing 99.92% and 94.74% of the oils respectively. Oxygenated monoterpenes were found to be the major group in both **P-1** (99.47%) and **P-2** (89.88%). **P-1** consists almost entirely of *p*-Menth-6-en-2-one (Carvotanacetone, 98.59%). **P-2** is characterized by high contents of each of Dimethoxydurene (38.48%), Durenol (26.89%) and 2′,4′-Dimethoxy-3′-methylacetophenone (20.52%). Both oils showed moderate antimicrobial activity against the Gram-positive strains and *C. albicans.* However, no activity was shown against Gram-negative bacteria. **P-1** showed a significant cytotoxic activity against both MCF-7 and HEPG-2 (IC_{50} = 3.8 and 5.1 μg/ml, respectively), while **P-2** showed selective cytotoxic activity against MCF-7 cell line (IC_{50} = 9.3 μg/ml). **Conclusion:** The potent cytotoxic and moderate antimicrobial activities of **P-1** may be attributed to its high content of Carvotanacetone.

Keywords: Carvotanacetone, cytotoxicity, essential oil, *Pulicaria jaubertii*

INTRODUCTION

Genus *Pulicaria*, belonging to the tribe Inuleae of the Asteraceae family, consists of *ca*. 100 species distributed in Europe, North Africa and Asia.[1] The genus is represented in Saudi Arabia by eight species. *Pulicaria jaubertii* Gamal-Eldin [syn. *Pulicaria orientalis* Jaub.] is a perennial fragrant herb with erect branches up to 50 cm high. It is known in Arabic as "Eter Elraee".[2] The *Pulicaria* species proved various activities such as anti-inflammatory, antileukemic,[3] potential cancer chemopreventive and cytotoxic agents.[4] Previous investigations reported that *P. Jaubertii* showed antimicrobial, antifungal, antimalarial and insecticidal properties.[5] Different species of *Pulicaria* have been studied to establish the composition of their essential oils.[6-9] In this study, we report and compare the results of GC-MS analyses of *P. jaubertii* leaf **(P-1)** and root **(P-2)** essential oils, as well as their *in vitro* antimicrobial and cytotoxic activities.

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MATERIALS AND METHODS

Plant material

P. jaubertii was collected in March 2011 from Jazan, South of Saudi Arabia. The plant was identified by Professor Mohammed Youssef, Department of Pharmacognosy, College of Pharmacy, King Saud University, where a voucher specimen (no. 15715 A) has been deposited.

Extraction of the essential oil

The freshly cut leaves and roots (400g of each) were separately subjected to hydrodistillation for 6h using a Clevenger-type apparatus according to the method

recommended in the European Pharmacopoeia.[10] The obtained oils were dried over anhydrous sodium sulphate and stored in air-tight, amber colored glass vials at 4°C.

Gas chromatography analysis

GC-MS analyses of the volatile oils were carried out using Focus GC/DSQ II mass spectrometer. The Column used was Trace TR-5 (30m × 0.25mm i.d., film thickness 0.25μm). Helium was used as carrier gas at flow rate of 1 ml/min. in split mode 20%. The oven program started with an initial temperature of 50°C for 1 min, and then it was raised to 250°C with 4°C/min. rate and finally held for 2 min. at this temperature. Kovat's retention indices were calculated using co-chromatographed standard hydrocarbons. The individual compounds were identified by comparing their retention indices relative to C8-C26 n-alkanes and by comparing their mass spectra and retention times with data already available in the NIST (National Institute of Standardization and Technology) library and literature.[11]

Determination of antimicrobial activity

Test organisms

The following strains of pathogenic microorganisms were used for the antimicrobial assay: *Bacillus subtilis* ATCC 26633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATTC 27853. The yeast strain used in this study was *Candida albicans* ATCC 10231. The microbial strains were obtained from American type culture collection (ATCC).

Broth micro-dilution assay for minimum inhibitory concentration (MIC)

The broth micro-dilution technique was used to determine the MIC values.[12] All of the experiments were performed in Mueller Hinton broth (Hi Media, Mumbai) for the bacterial strains and RPMI 1640 medium for the fungal strain. Two-fold serial dilution of the essential oils was prepared in a 96-well microtiter plate up to 2mg/ml. The prepared microtiter plates containing the microorganisms and the essential oils were then incubated at 37°C for 24h for bacterial growth and at 27°C for 48h for fungal growth. The growth of organisms was observed as turbidity, which was visually observed. Controls were set up with equivalent quantities of dimethyl sulfoxide 10% solution, which was used as a solvent for the essential oils. Amoxicillin, Gentamicin and Nystatin (Sigma, USA) were used as positive controls. All of the experiments were performed in triplicate.

Cytotoxicity assay

Cell culture

Mammalian cell lines: MCF-7 cells (human breast cancer cell line) and HEPG-2 (human liver cancer cell line), were obtained from VACSERA Tissue Culture Unit. The cells were propagated in Dulbeccos modified Eagles Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, Mo, USA), 1% L-glutamine, HEPES buffer and 50 μg/ml gentamycin (Sigma Chemical Co., St. Louis, Mo, USA). All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub-cultured two times a week.

Evaluation of cellular cytotoxicity

The cytotoxic activity was evaluated by the crystal violet staining (CVS) method described by Saotome *et al*[13] and modified by Itagaki *et al*.[14] Briefly, in a 96-well tissue culture microplate, the cells were seeded at a cell concentration of 1×10^4 cells per well in 100μl of growth medium. Fresh medium containing different concentrations of **P-1** and **P-2** were added after 24h of seeding at 37°C. Serial twofold dilutions of the tested oils were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells was found not to affect the experiment. After the 48 h incubation period, the viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using distilled water. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly. The quantitative analysis (colorimetric evaluation of fixed cells) was performed by measuring the absorbance in an automatic Microplate reader (TECAN, Inc.) at 595nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested oils. All experiments were carried out in triplicate. The effect on cell growth was calculated as the difference in absorbance percentage in presence and absence of the tested oils and illustrated in a dose-response curve. The concentration at which the growth of cells was inhibited to 50% of the control (IC_{50}) was obtained from this dose-response curve. The standard antitumor drug used was vinblastine sulfate.

Statistical analyses

Data were expressed as means ± S.D. For multi-variable comparisons, one-way ANOVA was conducted, followed by Tukey-Kramer testing using the GraphPad InStat (ISI Software) computer program. Differences were considered significant at P values of less than 0.05.

RESULTS AND DISCUSSION

Composition of the essential oils

Hydrodistillation of the leaves of *P. jaubertii* gave a pale yellow oil **P-1**, with a strong pleasant aromatic odor (yield 0.5% v/w), while hydrodistillation of the roots gave a dark yellow oil, **P-2** (yield 0.43% v/w). The chemical compositions of the investigated oils are presented in Table 1, where the identified components are listed in order of their elution on the Trace TR-5 column with their retention indices and percentages. A total of 16 volatile constituents were identified in **P-1**, while 23 components were identified in **P-2**, representing 99.92% and 94.74% of oils respectively. The results of the GC-MS analyses of the two oils revealed some important variations between them. Oxygenated monoterpenes were found to be the major group in both **P-1** and **P-2**, constituting 99.47% and 89.88% of the oils, respectively. **P-1** consists almost entirely of *p*-Menth-6-en-2-one (Carvotanacetone, 98.59%), which was also found to be the major constituent of the essential oils of *Pulicaria undulate*[15] and *Pulicaria mauritanica*.[16] **P-2** is characterized by high contents of each of Dimethoxydurene (38.48%), Durenol (26.89%) and 2′,4′-Dimethoxy-3′-methylacetophenone (20.52%). Both oils contain small percentages of monoterpenes, sesquiterpenes and oxygenated sesquiterpenes.

Table 1: Chemical composition of P-1 and P-2

Compound	RI*	Percentage P-1	Percentage P-2
α-Thujene	932	-	0.05
α-Pinene	940	0.05	0.05
Camphene	953	0.03	0.25
α-Phellandrene	1005	0.02	0.02
3-Carene	1012	0.07	-
p-Methylanisole	1019	-	4.11
Limonene	1031	0.05	-
m-Cymene	1084	-	0.34
Linalyl butyrate	1130	0.01	-
Camphor	1143	0.27	0.19
Menthone	1154	0.04	-
Isoborneol	1156	-	0.01
Borneol	1166	0.06	-
p-Cymen-8-ol	1183	-	0.62
Methyl chavicol	1195	-	2.19
Pulegone	1237	-	0.01
p-Menth-1(7)-en-2-one	1238	-	0.01
p-Menth-6-en-2-one (Carvotanacetone)	1245	98.59	0.14
4-Hydroxy-2-methylacetophenone	1271	-	0.01
Borneol acetate	1285	-	0.03
Thymol	1290	-	0.23
Dimethoxydurene	1295	-	38.48
2′,4′-Dimethoxy-3′-methylacetophenone	1312	0.37	20.52
Durenol	1319	0.13	26.89
Nerol acetate	1365	-	0.52
α-Ionone	1426	-	0.03
Cinnamic acid	1438	-	0.01
α-Sesquiphellandrene	1524	-	0.03
Caryophyllene oxide	1581	0.04	-
α-Cadinol	1653	0.01	-
14-Hydroxy-δ-cadinene	1799	0.01	-
1-Docosene	2195	0.17	-
Total percentages		99.92	94.74

*RI, retention indices relative to C8–C26 n-alkanes on the Trace TR-5 column

To the best of our knowledge this work represents the first GC-MS analysis of *P. jaubertii* root oil. Previous GC-MS study of *P. jaubertii* aerial parts oil,[6] confirmed the presence of oxygenated monoterpenes such as thujone and linalool, in addition to presence of mono- and sesquiterpenes but their percentages were not reported.

Antimicrobial activity

The antimicrobial activity of the investigated oils was evaluated by determining MIC values against two Gram-positive and two Gram-negative bacteria as well as against one fungal strain. The results of the assay are shown in Table 2. The results exhibited that the oils had varying degrees of growth inhibition against the Gram-positive strains and *C. albicans*. However, no activity was shown against Gram-negative bacteria. **P-1** demonstrated a higher antibacterial activity (MIC range 0.5-1 mg/ml) than **P-2** (MIC 2 mg/ml) against *Bacillus subtilis* and *Staphylococcus aureus*.P-1 showed antifungal activity against *C. albicans* at 1 mg/ml, while **P-2** did not show any antifungal activity.

Oxygenated monoterpenes were reported to be responsible for the antimicrobial activity of several essential oils.[17] Moreover the predominance of Carvotanacetone (98.59%) in **P-1** could contribute to the observed antimicrobial activity. It has been reported that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria.[18] Resistance of Gram-negative bacteria against essential oils has been attributed to the presence of a hydrophilic outer membrane containing a hydrophilic polysaccharide chain which acts as a barrier to the hydrophobic essential oil.[19]

In vitro antitumor evaluation

The antitumor activity of **P-1** and **P-2** against MCF-7 and HEPG-2 carcinoma cell lines, was determined using CVS method and vinblastine as a reference drug. The response parameter (IC_{50}) was calculated for each cell line [Tables 3 and 4].

P-2 showed a lower cytotoxic activity (IC_{50}= 9.3 and 18.3 μg/ml) than **P-1**, but it could be seen that both **P-1** and **P-2** showed concentration-dependent decrease in surviving fractions of MCF-7 and HEPG-2 cells. **P-1** is

Table 2: Antimicrobial activity of the investigated essential oils P-1 and P-2

Tested sample	MIC+				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
P-1	1000	500	r	r	1000
P-2	2000	2000	r	r	r
Amoxicillin	3.5	3.5	nt	nt	nt
Gentamicin	nt	nt	3.5	7	nt
Nystatin	nt	nt	nt	nt	3.5

+Mininmum inhibitory concentration values are given as µg/ml. nt: not tested. r: resistant at 2000 µg/ml of tested oil

Table 3: *In vitro* antitumor activities of P-1 and P-2 on MCF-7

Tumor cell line	Sample concentration (µg/ml)	Viability % ± S.D.#		
		P-1	P-2	Vinblastine sulfate (reference drug)
MCF-7	50	23.68 ± 1.64[a]	29.78 ± 2.25[a**]	7.82 ± 0.98
	25	35.12 ± 0.98[a]	37.44 ± 1.98[a]	15.18 ± 1.23
	12.5	41.36 ± 2.14[b]	45.26 ± 2.36[a]	29.26 ± 2.74
	6.25	46.93 ± 2.45	53.32 ± 3.12[b]	42.35 ± 2.21
	3.125	51.08 ± 1.88	59.9 ± 2.96[*]	56.54 ± 1.96
	1.56	58.22 ± 2.36[c]	67.62 ± 3.22[**]	67.24 ± 2.94
	0	100.00	100.00	100
++IC_{50}		3.8 µg/ml	9.3 µg/ml	4.6 µg/ml

#Mean of surviving fraction ± S.D.; mean of three assays ± standard deviation, [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$ compared to reference drug, *$P < 0.01$, **$P < 0.05$ compared to P-1 ++IC_{50} sample concentration required to inhibit tumor cell proliferation by 50%

Table 4: *In vitro* antitumor activities of P-1 and P-2 on HEPG-2

Tumor cell line	Sample concentration (µg/ml)	Viability % ± S.D.#		
		P-1	P-2	Vinblastine sulfate
HEPG-2	50	8.59 ± 1.64[c]	25.64 ± 2.16[a*]	14.38 ± 1.41
	25	14.28 ± 2.06	36.50 ± 2.50[a*]	16.13 ± 2.24
	12.5	27.62 ± 2.98	58.79 ± 1.96[a*]	24.25 ± 2.96
	6.25	43.35 ± 2.63	73.14 ± 2.38[a*]	45.13 ± 2.04
	3.125	61.50 ± 3.02[c]	84.71 ± 2.13[a*]	55.00 ± 2.33
	1.56	82.54 ± 2.24[b]	97.28 ± 2.04[a*]	72.13 ± 3.06
	0	100	100	100
++IC_{50}		5.1 µg/ml	18.3 µg/ml	4.6 µg/ml

#Mean of surviving fraction ± S.D.; mean of three assays ± standard deviation, [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$ compared to reference drug, *$P < 0.001$ compared to *P*-1.
++IC_{50} sample concentration required to inhibit tumor cell proliferation by 50%.

more potent as a cytotoxic agent, than **P-2**. It exhibited a significant cytotoxic activity against both cell lines. In case of MCF-7, IC_{50} of **P-1** (IC_{50}= 3.8 µg/ml) was less than that of the reference drug used (IC_{50} = 4.6 µg/ml) revealing its higher cytotoxic potency. As for HEPG-2 carcinoma cell lines IC_{50} of **P-1** (IC_{50}= 5.1 µg/ml) was close to that of the reference drug (IC_{50} = 4.6 µg/ml).

The potent cytotoxic effect of **P-1** may be attributed to its high content of Carvotanacetone, which was previously reported to have anticarcinogenic and chemopreventive activity.[20] **P-2** showed a selective cytotoxic activity against MCF-7 cell line (IC_{50}= 9.3µg/ml) compared with the reference drug (IC_{50} = 4.6 µg/ml). It seems from our results that the human breast cancer (MCF-7) cell line is the most sensitive to the studied essential oils.

CONCLUSION

To the best of our knowledge, this is the first report on either the chemical composition or bioactivity of the root essential oil and on the bioactivity of the leaf essential oil of *Pulicaria jaubertii*.

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