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RESEARCH PAPER

Chemical Compositions of Essential Oil of *Gailonia aucheri* from Iran and it's Antimicrobial and Antioxidant Activities and Total Phenol Contact

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Abstract The composition of the essential oil obtained from the dried flowering aerial parts of *Gailonia aucheri* (Rubiaceae) was analyzed by GC and GC–MS. Thirty-three components have been identified in the essential oil of *G. aucheri*. The major components of the essential oil were myristicin (32.8), oleic acid (9.2) and *trans*-ocimene (6.3%). The antimicrobial activity of *G. aucheri* oil was studied using the determination of minimal inhibitory concentration (MIC) values. The plant showed a moderate antimicrobial activity against the tested microorganisms. Best activities can be seen against *Staphylococcus epidermis* (Gram-positive bacteria), E*scherichia coli* (Gram-negative bacteria), and *Aspergillus niger* (fungi) with MIC values of 8, 16, and 8 mg/ml, respectively. In the DPPH radical scavenging assay, the plant oil showed the strong activities with IC_{50} values of 7.1 mg/ml. Antioxidant activity correlated well with the total phenolic content of the plant oil.

Keywords *Gailonia aucheri* · Essential oils · Antimicrobial activity · GC–MS · Myristicin

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1 Introduction

Since ancient times crude herbal extracts of aromatic plants have been in use for different purposes, such as food, drugs and perfumery (Doulah et al. 2014). In many developing countries, some plant materials play an important role in PHC (primary health care) (Sokmen et al. 1999). Some materials produced by aromatic plants such as essential oils have antimicrobial activity. Despite the chemical drugs with antimicrobial activity are developing, some herbal drugs are used for the prevention and treatment of infectious diseases in some countries (Federspil et al. 1997; Inouye et al. 2001). In the essential oil of aromatic plants, there are many compounds such as monoterpenes, sesquiterpenes, alcohols, aldehydes, phenols, esters, and ether, sulphurous and nitrogenous substances (Cowan 1999; Delamare et al. 2007).

Gailonia is a genus of flowering plants in the family Rubiaceae. The genus was expanded in 2007 when several other *Rubiaceae* genera were merged into it. There are now about 34 species. Plants of this genus are herbs or shrubs. They generally have an offensive scent when crushed. The leaves are oppositely arranged. The corolla is funnel shaped with a short to long tubular base. The fruit is either a drupe or a body that splits into two parts when ripe (Backlund et al. 2007). One of the most famous species of this genus with the largest geographical distribution is *G. aucheri* that grows wild in the south of Iran, Iraq and north of Oman (Batanouny and Ismail 1985). Its Persian name is "Kartoos" and in the South of Iran, different preparations of this plant such as decoctions, infusions and powders, are used as medicinal plant (Poor-Ramazan 2013).

The literature review showed a few examples of previous trials conducted on the biological properties of various *Gailonia* species (Durrani et al. 2010; Hakemi-Vala et al.

2014; Herrera et al. 1996). To the best of our knowledge, there are no literature reports to date concerning the volatile components of the aerial parts of *G. aucheri*. Our main aim here was thus to study the chemical composition of essential oils extracted from aerial parts of *G. aucheri* and determination of its antimicrobial activity.

2 Materials and Methods

2.1 Plant Material and Isolation of Essential Oil

The aerial parts of wild *G. aucheri* were collected from Bamdezh wetland (31.42N, 48.37E) in the northwest of Ahvaz, Iran, in July 2015 at full flowering stage. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist, Dr. M. Gholami, in the Department of Medicinal Plant, Jondi Shapour University of Medical Sciences, Ahvaz (Iran). The voucher specimen (JSA-213) has been deposited in the Herbarium Center at Jondi Shapour University, Ahvaz (Iran). Collected plant materials were dried in shade and ground in a grinder. The dried plant samples (285 g) were subjected to hydrodistillation (plant material in boiling water) using a Clevenger-type apparatus for 4 h.

2.2 Gas Chromatographic Analysis

Gas Chromatographic analysis was performed by using Hewlett-Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60 m × 0.32 mm. id) was used. The oven temperature was maintained initially at 50 °C for 5 min, then programmed from 50 to 250 °C at a rate of 4 °C/min. Helium was used as the carrier gas, at flow rate of 1.1 ml/min. The injector and detector temperatures were 220 and 250 °C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C8–C30, Aldrich Co.) as references.

2.3 Gas Chromatography–Mass Spectrometry

The oil was analyzed using a Hewlett-Packard gas chromatograph Model 6890 coupled to a Hewlett-Packard MS Model 5973 equipped with an HP5 column (30 m × 0.25 mm, film thickness 0.25 μm). The oven temperature was programmed from 60 (5 min) to 220 °C at 5 °C/min, and 5 min hold. The carrier gas was helium (1.0 mL/min), injection was set in the split mode (1/10). Identification was carried out by comparing their retention indices (RI), with NIST (National Institute of Standards and Technology) library and by comparing their mass spectra with data already available in the literature (Adams 2012).

2.4 Antimicrobial Activity

Six strains of bacteria and two strains of fungi were studied for including *Klebsiella pneumonia* (PTCC 1290), *Staphylococcus aureus* (PTCC 1431), *S. epidermidis* (PTCC 1435), *Bacillus cereus* (PTCC 1665), *Pseudomonas aeruginosa* (PTCC 1707), *Escherichia coli* (PTCC 1395), *Penicillium digitatum* (PTCC 5251), *Aspergillus niger* (PTCC 5012). Bacterial strains were cultivated on Mueller–Hinton broth (Merck, Germany) for 24 h at 37 °C and fungi were cultivated on Sabouraud liquid medium broth (Merck, Germany) at 25 °C for 48 h to 3 days before use. All microorganisms were maintained at −20 °C under appropriate conditions and regenerated twice before use in the manipulations. The minimum inhibitory concentration (MIC) of essential oil and extracts against the bacteria and fungi was determined using micro-well dilution assay method as described previously (Ebrahimabadi et al. 2010). The inocula of the microbial strains were prepared from 12-h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oil of *G. aucheri* dissolved in 10% DMSO was first diluted to the highest concentration (512 μg/ml) to be tested, and then serial twofold dilutions were made in a concentration range from 4 to 512 μg/ml in 10 ml sterile test tubes containing brain heart infusion (BHI) broth for bacterial strains and Sabouraud dextrose broth (SDB) broth for yeast. The 96-well plates were prepared by dispensing 95 μl of the cultures media and 5 μl of the inoculum into each well. A 100 μl aliquot from the stock solutions of the essential oil initially prepared at the concentration of 500 μg/ml was added into the first well. Then, 100 μl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 μl of the cultures media without the test materials and 5 μl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 μl. Ampicillin, tetracycline and fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by the presence of a white pellet on the well bottom and confirmed by plating 5 μl samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the plant extracts required for inhibiting the growth of microorganisms. Each test was repeated at least twice.

Table 1 Chemical components identified from the essential OIL of *G. aucheri*

No.	Compound	KI[a]	%	KI/MS
1	Propyl-cyclopentane	880	0.5	KI/MS
2	*cis*-4-Decene	905	3.8	KI/MS
3	Isopropylidenecyclopropyl tert-butyl ether	910	1.0	KI/MS
4	5-Methyl-1-hexene	911	1.0	KI/MS
5	5-Methyl-4-nonene	917	3.8	KI
6	Sabinene	919	0.4	KI/MS
7	5-Methyl-4-nonene	921	5.3	KI/MS
8	1-Decene	924	2.5	KI/MS
9	*trans*-5-Decene	925	2.4	KI/MS
10	Decane	927	0.5	KI
11	*cis*-Ocimene	1004	2.6	MS
12	*trans*-Ocimene	1007	6.3	MS
13	(*E*)-6-Dodecene	1105	0.5	KI/MS
14	5-Methyl-5-undecene	1109	0.4	KI/MS
15	1-Methyl-2-(4-methylpentyl)cyclopentane	1109	0.4	KI/MS
16	1-Dodecene	1113	2.0	KI/MS
17	1-Pentadecene	1300	1.3	KI/MS
18	Methyleugenol	1384	1.2	KI/MS
19	α-Guaiene	1389	0.3	KI/MS
20	α-Farnesene	1395	0.4	KI/MS
21	Isoeugenyl methyl ether	1396	0.3	KI/MS
22	α-Bulnesene	1482	0.4	KI/MS
23	Myristicin	1484	32.9	KI/MS
24	8a-Methylhexahydro-1,8(2H,5H)-naphthalenedione	1484	0.5	KI/MS
25	1-Heptadecene	1491	0.8	KI/MS
26	β-Maaliene	1585	0.7	KI/MS
27	Heneicosane	1781	1.4	KI/MS
29	Palmitic acid	1876	1.9	KI/MS
29	Isobutyl phthalate	1968	0.6	KI/MS
30	Docosane	1976	0.6	KI/MS
31	7-Pentadecyne	2070	2.8	KI/MS
32	Oleic acid	2070	9.2	KI/MS
33	Brassylic acid	2173	1.1	KI/MS
Total			90.5	

[a] Kovat's retention index

2.5 DPPH Radical Scavenging Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay usually involves hydrogen atom transfer reaction, but, based on kinetic data, an electron transfer mechanism has also been suggested for this assay (Foti et al. 2004; Huang et al. 2005). Radical scavenging activity (RSA) of *G. aucheri* essential oil was measured against the stable free radical DPPH as described previously (Brand-Williams et al. 1995). Briefly, three different dilutions of essential oil, in the range 2.5–20 mg/ml, were incubated with a methanolic solution of DPPH 100 µM. After 30 min of incubation at room temperature, the absorbance at 517 nm was measured by a spectrophotometer (Bio-Tek, Model Uvikon XL). The percentage of inhibition (*%I*) of the radical was calculated according to the change of absorbance of the DPPH solution for each dilution of essential oil and IC_{50} values were determined.

2.6 Total Phenolic Content

Total phenolic content in *G. aucheri* essential oil was determined by the Folin–Ciocalteau colorimetric method as described previously (Firuzi et al. 2010). Briefly, 10 µl of essential oil with 10 µl of Tween-20 were mixed with 0.5 ml Folin–Ciocalteau reagent diluted ten times in

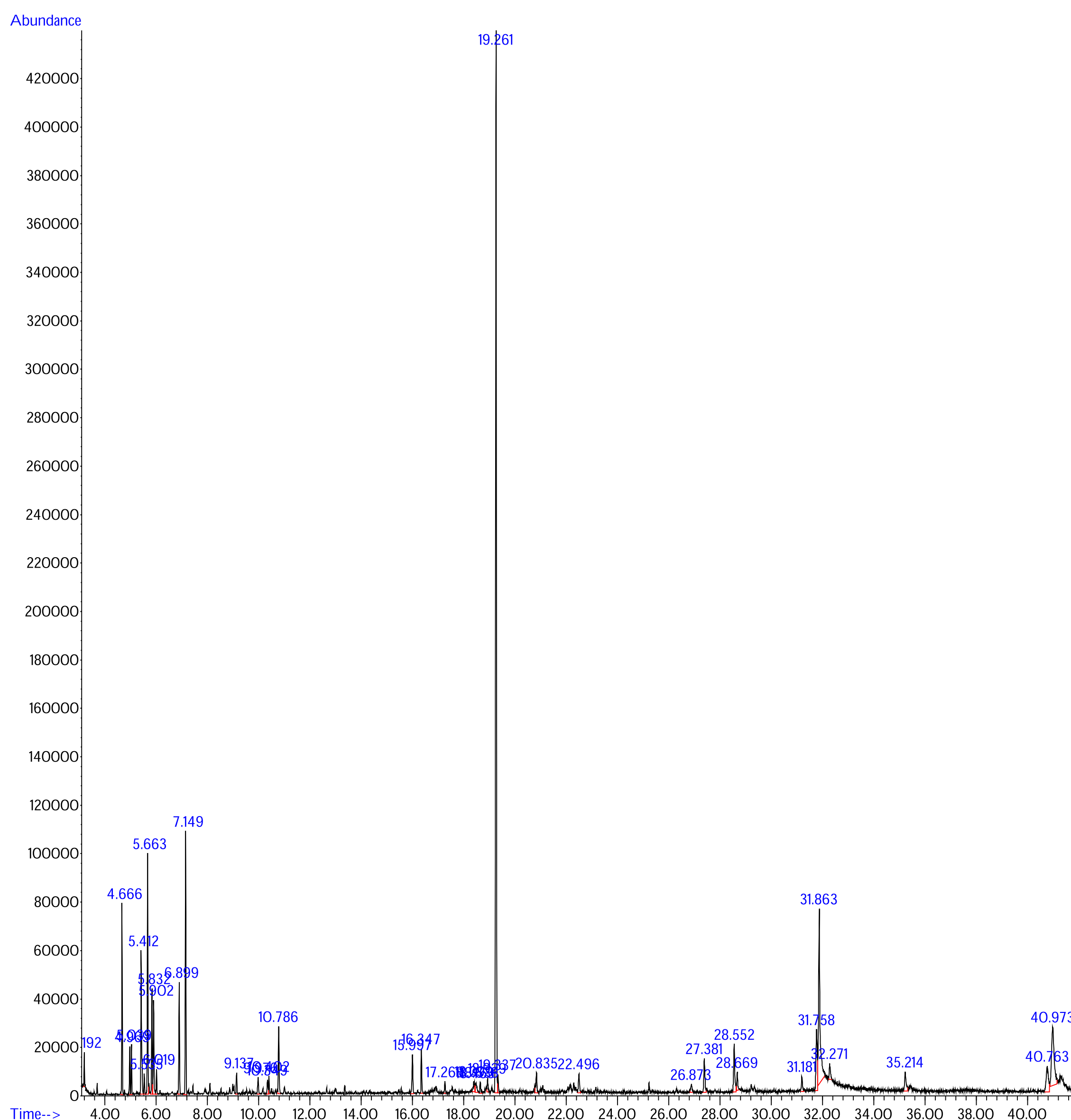

Fig. 1 GC total ion chromatogram of *G. aucheri* essential oil

deionized water. A methanolic solution of catechin 0.25 mg/ml was tested in parallel as reference compound. After 5 min of incubation at room temperature, 0.4 ml of Na_2CO_3 7.5% in water was added to the samples and they were incubated at room temperature in the dark. The absorbance at 760 nm was read after 90 min against a blank of deionized water with a spectrophotometer (Bio-Tek, Model Uvikon XL). The total phenolic content was expressed as mg of catechin equivalent in each g of essential oil.

2.7 Statistical Analysis

Multiple comparisons among antioxidant and total phenol values were performed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test using the software SPSS (version11.5.0 for Windows; SPSS Inc., Chicago, IL). Data were considered statistically different at $P < 0.01$.

3 Results and Discussion

The hydrodistillation of the dried aerial parts of *G. aucheri* gave light yellowish oil with yield of 0.8% w/w (2.3 g). The chemical compositions of essential oil of aerial parts of this plant are shown in Table 1. Thirty-three components, representing 90.5% of the total components in the oil, have been identified in the essential oil extracted from the aerial parts of this plant. The GC total ion chromatogram of plant

Table 2 Antimicrobial activity of *G. aucheri* essential oil

Test	Microorganisms	Essential oil (μg/ml)	Standard[a] (μg/ml)
Gram-negative bacteria	*Klebsiella pneumoniae*	512	4
	Pseudomonas aeruginosa	256	16
	Escherichia coli	16	4
Gram-positive bacteria	*Staphylococcus aureus*	32	8
	Staphylococcus epidermidis	8	4
	Bacillus cereus	32	16
Fungi	*Aspergillus niger*	8	4
	penicillium digitatum	128	8

[a] Ampicillin, tetracycline and fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively

essential oils is shown in Fig. 1. The major components of the essential oil were myristicin (32.8), oleic acid (9.2) and *trans*-ocimene (6.3%). As shown in the Table 1, this oil is rich in medicinal compound myristicin. This compound is a phenylpropene, insoluble in water, but soluble in ethanol and acetone. Myristicin is a psychoactive drug, acting as an anticholinergic, and is the traditional precursor for the psychedelic and empathogenic drug (Shulgin et al. 1967).

According to the data shown in Table 2, the essential oil of *G. aucheri* had moderate antimicrobial activity against all tested microorganisms. The best antibacterial activity of essential oil of *G. aucheri* was observed against *S. epidermis* (Gram-positive bacteria), *E. coli* (Gram-negative bacteria), and *A. niger* (fungi) with MIC values of 8, 16, and 8 mg/ml, respectively. Overall the essential oils possessed stronger antimicrobial activity against Gram-positive bacteria than against Gram-negative bacteria. According to the literature, various plant essential oils containing a relatively high proportion of oxygenated monoterpenes show better antimicrobial activities against Gram-positive bacteria (Kordali et al. 2005; Sacchetti et al. 2005; Tuberoso et al. 2005). The results indicate that *G. aucheri* may become important in obtaining a noticeable source of compounds with health protective potential, antioxidant and antimicrobial activity. It showed mildly significant activity against Gram-positive and Gram-negative bacteria, and fungi.

The essential oil demonstrated good antioxidant activities. IC_{50} for DPPH radical-scavenging activity was 7.1 ± 0.6 mg/ml. The IC_{50} values for quercetin as a reference compound were 0.005 ± 0.001 mg/ml. The total phenolic content was 1.793 ± 0.002 mg catechin equivalent/g essential oil. A good linear correlation was observed between the IC_{50} values in the DPPH assay and the total phenolic content (R^2: 0.819, data not shown). Myristicin is a good antioxidant (Zheng et al. 1992) and it could account for the high activity observed in *G. aucheri*.

4 Conclusion

According to literature data, this is the first study on the biological activity and chemical composition of the essential oil of *G. aucheri*. Our results show that *G. aucheri* has moderate antimicrobial activities, which is probably in part accounted for the presence of myristicin and also due to the synergism of various antimicrobial agents. Also *G. aucheri* showed good antioxidant activities, which could probably be in part due to the presence of myristicin and *trans*-Ocimene. These results encourage complementary and more in-depth studies on the chemical composition of the plant essential oils with the aim of separation and structure elucidation of their active components and evaluation of biological activity of each compound separately. Also the considerable biological activities of *G. aucheri* essential oils make it A good candidate to develop natural-derived therapeutics.

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