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Chemical Composition and Antimicrobial Activity of *Pituranthos chloranthus* (Benth.) Hook and *Pituranthos tortuosus* (Coss.) Maire Essential Oils from Southern Tunisia

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Abstract

Essential oils (EO) from fresh and dry aerial parts of *Pituranthos chloranthus* (Benth.) Hook and *Pituranthos tortuosus* (Coss.) Maire were isolated by hydrodistillation and analyzed by GC/MS. The main constituents of the EO obtained from fresh herb of *P. chloranthus* were found to be *α*-pinene, sabinene, *cis*-ocimene and myrcene. In dry biomass, a significant increase of the content of some compounds such as *α*-phellandrene, Δ,3-carene and *β*-phellandrene characterized the oil. Minor changes in the chemical composition of the *P. tortuosus* EOs obtained from fresh or dry herbs and the major constituents were found to be sabinene and myrcene with equilibrate amounts of *α*-pinene, *p*-cymene, *cis*-ocimene, limonene, *trans*-*β*-ocimene, *γ*-terpinene and *cis*-verbenol. The paper disc diffusion method was used to evaluate the antibacterial activity and results showed an important inhibitory effect of oils obtained from fresh herb against most tested bacteria.

Keywords

***Pituranthos*, *Chloranthus*, *Tortuosus*, Essential Oil, South Tunisia**

1. Introduction

Pituranthos chloranthus (Benth.) Hook (Apiaceae) is an endemic and aromatic plant, locally named "Aljène" which grows naturally in North Africa and is widespread in central and southern Tunisia. Stems of this plant have been traditionally used as straw for farmers to dry figs and grapes. This plant has a double advantage: first,

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it has been used for its aroma and distinctive taste that adhere to the dry fruits; second, it has an insecticidal effect. In the southern Tunisian, the tuft of *P. chloranthus* was, traditionally, suspended to the surface of the water to disinfect the underground cisterns of the rain water storage used for the human drink. Furthermore, *Pituranthos* species are used in traditional medicine for the treatment of asthma, rheumatism, postpartum care, spasms, pains, fevers, diabetes, lice (head and pubis), hepatitis, digestive difficulties, urinary infections and scorpion's stings [1].

Previous studies have shown an interest changes in the chemical composition of EOs of *P. chloranthus*. Indeed, from distinct geographical areas in Tunisia and at various development stages of this species, the aerial part contains various oils types mainly composed of *α*-pinene, *β*-pinene, *α*-phellandrene, *β*-phellandrene, *β*-myrcene, *p*-cymene, 8-methyldecanal, exo-2-hydroxycineole acetate, carvacrol, geraniol and *β*-damascenone [2]. These oil types were found to exhibit antibacterial, antioxydant and anti-genotoxic activities [2] [3]. Yanghui *et al*. [1] demonstrated that *P. chloranthus* EO collected at Sfax (Tunisia) was mainly composed of terpinen-4-ol, 8-hydroxy-*p*-cymene, myrtenol, p-menth-2-en-1-ol and *α*-terpineol and exhibited antioxidant, antifungal and insecticidal activities suggesting its potential use as an alternative natural agent for disinfection.

Pituranthos tortuosus (Coss.) Maire (Apiaceae), locally named as "Guezzah", is a small shrub without leaves. This aromatic plant, which grows naturally in North Africa, is also, widespread in central and southern Tunisia. The plant is used by the Egyptians for the preparation of carminative drink and is occasionally eaten by grazing animals [4] [5]. It is also used for relief of stomach pains, against intestinal parasites, when blood is excreted in the urine or when coughing blood, and for the regulation of menstruation [4] [5]. In Tunisia, *P. tortuosus* is used traditionally as an anti-asthmatic and against scorpion's stings [5]. The composition and biological activities of *P. tortuosus* EO have been previously reported [4]-[8]. Abdewahed *et al.* [6] show that the chemical composition and the bacterial activity of EO isolated from fresh aerial parts collected in Monastir (center of Tunisia) depend on the period of collect: the November EO mainly composed of myrtenol is more effective than that of April mainly composed of terpinen-4-ol against the Gram-positive bacteria *Enterococcus faecalis* and *Staphylococcus aureus*. These oils also showmutagenic, antimutagenic, cytotoxic and apoptotic activities [7]. EO is isolated from dried aerial parts of this species collected at Sousse showed strong insecticidal, antifungal and allelopathic activities [5]. In Egypt, Hossam *et al.* [8] report that the composition and anticancer activity of EO isolated from aerial parts depend on the method of extraction: the simultaneous hydrodistillation-solvent (*n*-pentane) extraction method shows the most potent activity against the three human cancer cell lines tested (liver, colon and breast cancer cell lines). The EO from southern Sinai, contained 32 components with camphene (31.0%) as the major constituent, is ineffective against Gram-positive bacteria [4].

Never previously studies have been compared these two type of oils isolated from fresh and dried aerial parts of these endemic plants from southern region of Tunisia. The purpose of this present study is to compare the oil yields, chemical composition and the antibacterial activity of these oils which attract the interest of the local farmers and/or EO producers in arid zones of Southern Tunisia.

2. Materials and Methods

2.1. Plant Material

Aerial parts of *P. tortuosus* were collected from Beni-khedech (Medenine) and *P. chloranthus* from Douiret (Tatouine) just before the flowering stage. These two areas belong to Matmata's mountainous chain in southern Tunisia. The collected biomass were divided into two groups, one used as fresh herb and the other air-dried during 20 days in shade at ambient temperature for the EO extraction with a modified Clevenger-type apparatus for 4 h. The EOs were collected, dried by anhydrous sodium sulfate and stored at 4˚C in tight vials until analysis.

2.2. GC and GC-MS Identification

GC analysis was carried out using an Agilent 6890N Network GC system gas chromatograph fitted with flame ionization detector (FID) and an electronic integrator, using a HP-5 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 mm). The oven temperature was programmed from 50˚C - 280˚C at 7˚C/min; injector temperature: 220˚C; detector temperature: 240˚C; carrier gas: nitrogen (1.0 mL/min); sample manually injected: 0.2 mL. Retention indices (RIs) were determined relative to the retention times of a series of n-alcanes (C_6 - C_{22}). The relative amount of components in the oil was calculated by electronic integration of FID peak areas and normalized without the use of response factor correction.

EOs constituents were also analyzed by GC-MS using the Agilent 6890N Network GC system combined with Agilent 5975 B Inert MSD detector (quadrupole) with electron impact ionization (70 eV). A HP-5-MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 mm) was used. The column temperature was programmed to rise from 50˚C - 280˚C at rate at 7˚C/min. The carrier gas was helium adjusted to a linear velocity of 34 cm/s. Scan time and mass range were 2.2 s and 50 - 550 *m/z*, respectively. Samples (0.1 mL) were injected with a split ratio of 1:100.

Identification of the components was based: 1) on comparison of their GC RIs on apolar column (HP-5) with those of literature data [6]-[9]. 2) by comparison of their recorded mass spectra with those of a computer library (Wiley 275 library and NIST98 database/Chem Station data system) provided by the instrument software and MS literature data [9] [10]; 3) identities of some other components were further confirmed by co-injection of pure standards available in the laboratory under the same GC/MS conditions as above.

2.3. Antibacterial Testing

The antibacterial activity of the different EOs was evaluated by the paper-disc agar diffusion method [11] against the *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (ATCC 25922) and *Klebsielle pneumoniae* (ATCC 3583). These clinical strains were obtained from Microbiology and Immunology Laboratory (EPS Habib Bourguiba, Medenine, Tunisia). Microorganisms were maintained on Muller-Hinton agar (MH) (BIORAD) medium. Inocula were prepared by diluting overnight (24 h at 37˚C) cultures in Muller Hinton Broth medium to approximately 106 CFU/mL. Absorbent discs (Whatman N˚3 discs, 6 mm diameter) were impregnated with 10 µL of oil and then placed on the surface of inoculated plates (90 mm). Positive control discs of gentamicin (10 µg/disc) were included in each assay. Diameters of growth inhibition zones were measured after incubation at 37˚C for 24 h.

3. Results and Discussion

The distilled EOs from fresh and dry biomass of *P. chloranthus* and *P. tortuosus* species collected in two localities from the Matmata's mountainous chain in southern Tunisia, were yellow. Air-drying the plant material in shade at ambient temperature (17˚C - 20˚C) not only resulted in a decrease of the *P. chloranthus* and *P. tortuosus* EO yields (1.6 and 2.0 from the fresh herb and 0.9 and 1.1 from the dry herb, respectively) but affected the density of these oils (0.8922 and 0.9216 of the oils obtained from the fresh herb and 0.7123 and 0.8931 from the dry herb, respectively). The refractive indexes of oils were similar ranging from 1.4987 to 1.4840 and 1.5000 to 1.4826, respectively (**Table 1**).

To determine the difference between analyzed oil samples, only sixteen major volatile compounds (amount > 1%) accounting for 93.3% to 97.2% of different oil types were retained as shown in **Table 1**. Qualitatively, the chemical composition remains stable between the two species belonging to the same genus regardless the biomass status. The main constituents of the EO obtained from fresh herb of *P. chloranthus* were found to be *α*-pinene (47.4%), sabinene (15.0%), *cis*-ocimene (6.6%) and myrcene (6.6%). In dry biomass, the amount of *α*-pinene decreases to 32.5% and *cis*-ocimene is present as trace but a significant increase of the content of some compounds such as *α*-phellandrene (3.8% to 7.8%), Δ,3-carene (trace to 5.7%) and *β*-phellandrene (3.4% to 13.9%) characterize this oil. Minor changes in the chemical composition of the *P. tortuosus* EO obtained from fresh or dry herbs and the major constituents were found to be sabinene (35.8% - 38.9%) and myrcene (8.9% - 12.5%) with equilibrate amounts of *α*-pinene, *p*-cymene, *cis*-ocimene, limonene, *trans*-*β*-ocimene, *γ*-terpinene and *cis*-verbenol (from 3.9% to 8.8%). The only compound that its content has been modified after drying the biomass is 3-n-butyl phthalide from 5.0% to 1.5%.

The influence of drying the aboveground biomass on the chemical composition has been previously reported by several authors [12]-[17]. A differential response of the aromatic species is attributed generally, to the loss of some compounds during the storage of the biomass after deteriorating oil glands and/or due to some physiological process that continue even after harvesting. The results of this work are in agreement with those obtained with others species confirming these qualitative and quantitative changes in the EO isolated from fresh and dried plant materials.

The composition of these EO isolated from our samples seems to be different from other regions in Tunisia and other countries. In fact, Yanghui *et al.* [1] demonstrated that the EO of *P. chloranthus* collected from Sfax (Tunisia) was mainly composed of terpinen-4-ol (30.3%), 8-hydroxy-*p*-cymene (4.2%), myrtenol (4.1%),

Table 1. Major compounds of the essential oils of *Pituranthos choloranthus* and *Pituranthos tortuosus.*

			Pituranthos chloranthus		*Pituranthos tortuosus*		
			Fresh herb	Dry herb	Fresh herb	Dry herb	
		Yield %	1.64	0.85	2.03	1.06	
Major		Refractive index	1.4987	1.4840	1.5000	1.4826	Identification
Compounds	Ri_{lit}	*Ri*	Density	0.8922	0.7123	0.9216	0.8931	Methods
α-pinene	937	942		47.4	32.5	6.2	5.1	A, B
Sabinene	976	964		15.0	12.6	38.9	35.8	A, B
β-pinene	980	989		2.0	1.7	1.8	2.1	A, B
Myrcene	986	993		6.6	4.6	8.9	12.5	A, B
α-phelandrene	1008	996		3.6	7.7	0.8	1.8	A
Δ-3-carene	1010	1005		tr.	5.7	2.9	1.9	A, B
p-cymene	1023	1011		7.5	6.4	4.9	8.8	A, B
Limonene	1031	1018		tr.	tr.	4.3	5.0	A, B
β-phellandrene	1032	1026		3.4	13.9	1.3	1.0	A
cis-*β*-ocimene	1037	1037		6.6	tr.	5.6	6.6	A, B
trans-*β*-ocimene	1045	1041		0.4	0.9	5.0	3.9	A, B
γ-terpinene	1058	1070		0.5	0.9	4.3	5.5	A, B
cis-verbenol	1140	1149		0.7	0.7	6.1	5.1	A
t-cadinol	1640	1625		1.5	1.8	tr.	0.7	A, B
β-eudesmol	1649	1667		tr.	1.4	tr.	tr.	A
3-n-butyl phthalide	1720	1723		2.0	2.7	5.0	1.5	A

Ri_{lit}: Retention indexes on HP-5 column according to literature data; *Ri*: Retention index on a HP-5 column; tr.: trace (less than 1%); A: GC-GC/MS; B: co-injection with authentic standard.

p-menth-2-en-1-ol (4.0%) and *α*-terpineol (3.5%). EOs isolated from *P. chloranthus* harvested at the vegetative, flower budding, flowering and fruiting stages from three different areas of southern Tunisia (Gabès, Médenine and Benguerdane) were found to be mainly composed by *α*-pinene, *β*-pinene, *α*-phellandrene, *β*-myrcene, *β*-phellandrene, *p*-cymene, 8-methyldecanal, exo-2-hydroxycineole acetate and carvacrol (more than 10% for each compound) [2]. However, this composition varied with respect to both the geographical area and the season: *p*-cymenene was only detected at the floral budding stage (February), whereas high amounts of exo-2-hydroxycineole and exo-2-hydroxycineole acetate were specific for the flowering period (April). Carvacrol was shown to be characteristic of the fruiting period (August), whereas the vegetative stage (November) could be distinguished by the presence of *α*/*β*-pinene, limonene, camphene, geraniol and *β*-damascenone [2]. This difference on composition between oils suggests that different chemotypes of *P. chloranthus* exist in Tunisia. *P. tortuosus* oil types extracted from the fresh herb collected in Monastir (central Tunisia) depends on the period of the collect of vegetal samples [6]: EO of samples collected during November was mainly composed of myrtenol (26.2%), sabinene (11.0%), limonene (10.9%), *α*-pinene (5.5%) and 3-*n*-butyl phthalide (5.9%) whereas the oil of samples harvested during April was mainly composed of terpinen-4-ol (39.6%), 3-*n*-butyl phthalide (11.4%), butylidene phthalide (4.1%), limonene (4.1%) and sabinene (3.2%). The yield and the composition of EOs isolated from aerial parts of *P. tortuosus* growing wild in Egypt vary with the method of extraction [8]: the major components of the oil prepared by hydrodistillation were *β*-myrcene (18.8%), sabinene (18.5%), *trans*-*iso*-elemicin (12.9%), and terpinen-4-ol (8.1%); those predominant in the oil extracted by the simultaneous hydrodistillation-solvent (*n*-pentane) extraction were terpinen-4-ol (29.7%), sabinene (7.4%), *γ*-terpinene (7.3%) and *β*-myrcene (5.5%); while the prominent ones in the oil isolated by the conventional volatile solvent extraction sample were terpinen-4-ol (15.4%), dillapiol (7.9%), and *allo*-ocimene (4*E*, 6*Z*) (6.0%). The volatile oil of *P. tortuous* from Southern Sinai was found to have a different composition from other regions in Egypt with camphene (31.0%) as the major constituent [4]. This difference on composition between oils suggests that different chemotypes of *P. tortuosus* exist in Tunisia and around the world.

Table 2. Antimicrobial activity of the investigated essential oils and the standard antibiotic (gentamicin) against five bacteria (Inhibition zone diameters: mm).

	Gentamicin	*Pituranthos chloranthus*		*Pituranthos tortuosus*	
		Fresh herb	Dry herb	Fresh herb	Dry herb
Streptococcus pyogenes	40	30	12	27	20
Staphylococcus aureus	30	35	14	15	15
Enterobacter aerogenes	20	10	0	30	9
Escherchia coli	20	9	0	8	10
Klebsielle pneumoniae	15	7	8	10	6

The antimicrobial activities of *P. chloranthus* and *P. tortuosus* EOs collected from southern Tunisia were evaluated by a paper disc diffusion method against some bacteria. As shown in **Table 2**, results revealed that oils obtained from fresh herb (especially *P. chloranthus* oils) exhibited higher antibacterial activity than those of dried herbs. Indeed, concerning *P. chloranthus* oils, all tested bacteria were found to be more sensitive against the oil isolated from fresh herbs than that extracted from dried herbs except *K. pneumoniae* which showed the same weak activity with both type of oils (from fresh and dried herbs). Some strains, such as *E. aerogenes* and *E. coli*, which were resistant against dried herbs oil, showed a weak activity against fresh herbs oil. The highest activity has been observed for *S. pyogenes* and *S. aureus* with fresh herb oil (30 and 35 mm respectively) while dried herbs oil revealed a weak activity against these two strains (12 and 14 mm). These results demonstrated that the EO isolated from fresh *P. chloranthus* exhibited a potent antibacterial activity against *S. pyogenes* and *S. aureus* bacteria which was similar as 10 µg of gentamicin (standard antibiotic). This important activity could be attributed to the high amount of *α*-pinene (47.4%), sabinene (15.0%), myrcene (6.6%) and *cis*-*β*-ocimene (6.6%) known to have exhibit a potent activity against these stains. Minor components such as *γ*-terpinene and *cis*-verbenol could also contribute to this activity in the synergism with major components [18]-[21].

Concerning *P. tortuosus* EOs, except *E. aerogenes* which exhibited a significant difference on sensitivity against EO isolated from fresh and dried herbs (30 and 9 mm respectively), other strains were not affected by the type of the oil. *P. tortuosus* oils exhibited a weak activity against *E. coli* and *K. pneumoniae*, moderate activity against S. aureus and important activity against *S. pyogenes* and *E. aerogenes* (especially fresh herb oil for this last strain). These results showed that EO isolated from fresh *P. tortuosus* could be a potent antibacterial natural product, similar as 10 µg of gentamicin, against *E. aerogenes* bacteria. The important activity oft the fresh *P. tortuosus* oil against *S. pyogenes* and *E. aerogenes* could be attributed to its high content of sabinene (38.87%), *cis*-verbinol (6.09%) and 3-n-butyl phthalide (5.00%) (16 - 23). Some other components such as *cis*-*β*-ocimene, *trans*-*β*-ocimene, limonene and *γ*-terpinene could be also contribute to the antibacterial activity of this EO [18]-[21].

4. Conclusion

Our results have shown that air-drying aboveground biomass of *P. choloranthus* and *P. tortuosus* in shade at ambient temperature results in a decrease of the yield of EO but conversely, the drying time of the plant material has no effect on the qualitative oil composition. For both species, the oil isolated from fresh aerial parts could be revealed a potent antibacterial activity against *S. pyogenes*, *S. aureus* and *E. aerogenes* and have potential to be used as natural antibacterial agents for these types of bacteria. For this purpose, further studies should be undertaken on these EOs, especially those extracted from dried herbs, which could exhibited other biological activities due to their chemical composition which is different from fresh herbs.

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