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Phytochemistry and Antioxidant Activities of *Pelargonium denticulatum* Jacq. Essential Oils

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Abstract:
Background*: The active principles to which medicinal plants most often owe their properties belong to the category of substances that are usually classified today among "secondary metabolites": alkaloids, glycosides, terpene essences or phenyl-propane derived essences. The study of the active principles of medicinal plants from arid zones shows that essential oils constitute the second major group of plant components from these regions. The present study consists of an exploration of essential oil yields, their chemical composition and their antioxidant activities in P. denticulatum aerial parts.*
Materials and Methods*: P. denticulatum was harvested during the flowering stage. Plant material was air dried then subjected to hydrodistillation in a Clevenger apparatus. Essential oil yields were expressed as percent of the dry material primary used. Their chemical composition was analyzed by GC and GC-MS using HP-5MS column. Biological activities of these essential oils were estimated by three different antioxidant tests: anti-DPPH test, Ferric Reducing Antioxidant Power, and β-Carotene bleaching test. All samples were analyzed in triplicates.*
Results*: P. denticulatum essential oils yield was moderate (0.07% of dry matter). However, they expressed interesting antioxidant activities, with a high ferric reducing power reflected by an EC_{50}=1.8 mg.mL^{-1}. Chemical investigation by GC-MS showed that they were dominated by sesquiterpene (hydrocarbons and oxygenated) and fatty acid esters, with hexyl butanoate, bicyclogermacrene, and nerolidol as major compounds.*
Conclusion: *P. denticulatum essential oils are dominated by sesquiterpenes and fatty acid esters. They express respectable antioxidant activities, essentially as ferric reducing agents.*
Key Word*: Pelargonium denticulatum; Essential oils; GC-MS; Sesquiterpenes; Antioxidant activities.*

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I. Introduction

Since the Middle Ages, essential oils (EOs) have been used for their various properties (bactericidal, virucidal, antiparasitic, insecticidal and medicinal). Nowadays, they are mainly known for their use in the pharmaceutical, sanitary, cosmetic and food industries. Because of their extraction methods, generally by distillation from aromatic plants, these oils contain a variety of volatile molecules such as terpenes, terpenoids, aromatic compounds derived from phenols and aliphatic compounds[1]. The recent trend of using natural compounds in medicines and food preservation has led to an increasing interest in these secondary metabolites application. EOs and their components possess many biological properties such as antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties[1,2,3].

P. denticulatum is an aromatic plant widely distributed in the Mediterranean basin and in South Africa. Some previous studies have described the chemical composition and antibacterial activities of its essential oils[4]. However, no previous study, to our knowledge, was interested in the antioxidant potential of the essential oil in this species, which constitutes an originality of our work.

II. Material And Methods

Plant sampling

P. denticulatum was collected in the region of Soliman (governorate of Nabeul) in the North-East of Tunisia (N36°42', E10°29'), belonging to the Upper Semi-Arid bioclimatic stage (average temperatures 18°C, annual rainfall between 500-600 mm)[5]. The harvested plant was identified at the Biotechnology Center of Borj Cedria. A voucher specimen (PD-CBBC-07) was deposited in the herbarium of the Laboratory of Aromatic and Medicinal Plants (LPAM). Aerial parts were air dried at the shadow then grounded.

Essential oils isolation

Samples of 200g of *P. denticulatum* grounded aerial parts were subjected to hydrodistillation in 2L of deionized water using a Clevenger apparatus for 4 hours. Obtained essential oils were dried over anhydrous sodium sulphate and stored at +4 °C until tested. EO yields were expressed as percent of the plant material weight used. Extractions were performed in triplicates.

Gas chromatography (GC) analysis

GC analysis was carried out using an Agilent 6890 gas chromatograph equipped with a flame ionisation detector and split-splitless injector attached to HP-INNOWAX polyethylene glycol capillary column (30m x 0.25mm; 0.25µm film thickness). One micro-liter of the sample (dissolved in hexane as 1/50 v/v) was injected into the system. The constituents were identified by comparing their relative retention times with those of authentic compounds injected in the same conditions.

Gas chromatography/mass spectrometry (GC-MS) analysis of essential oils

Identification of the essential oils was performed using an Agilent 7890A GC-MS equipped with a HP5MS column (30m x 0.25mm x 0.25µm). Helium was used as carrier gas at 0.9 $mL.min^{-1}$. Each sample (1µL) was injected in the split mode (1:20), the program used was isothermal at 70 °C, followed by 50-240 °C at a rate of $5°C.min^{-1}$, then held at 240 °C for 10 min. The mass spectrometer was an Agilent 5975C.The total electronic impact mode at 70 eV was used. The components were identified by comparing their relative retention times and mass spectra with the data from the library of essential oils constituents, Wiley, Mass-Finder and Adams GC-MS libraries.

Antioxidant activities of *P. denticulatum* extracts and EOs

DPPH assay

DPPH quenching ability of plant extracts was measured according to Hanato et al.[6]. One mL of the EOs at different concentrations was added to 0.25 mL of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature in the dark. The DPPH radical-scavenging capacity was reported after 30 min reaction time for each diluted sample by the decrease in absorbance at 517 nm. IC_{50} value (mg. mL^{-1}), defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period, is a parameter widely used to measure antioxidant activity; a smaller IC_{50} value corresponds to a higher antioxidant activity of the plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = [(A0-A1)/A0]*100 (1)

Where A0 is the absorbance of the control at 0 min, and A1 is the absorbance of the sample at 30 min. All samples were analyzed in three replications.

FRAP assay

The iron (III) reductive capacity of the EOs was assessed as described by Oyaizu[7]. Briefly, 1mL of extract was mixed with 2.5 mL phosphate buffer (0.2 $mol.L^{-1}$, pH 6.6) and 2.5 mL (1 %) $K_3Fe(CN)_6$ solution. After 20 min at 50°C, 2.5 mL (10 %) trichloroacetic acid was added and the mixture was centrifuged for 10 min at 650 x *g*. Finally, a 2.5 mL aliquot was mixed with 2.5 mL ultra-pure water and 0.5 mL (0.1 g.100 mL-1) FeCl3 and the absorbance was recorded at 700 nm. Ascorbic acid was used as a positive control. A higher absorbance indicates a higher reducing power. Results are expressed as Effective Concentration at which the absorbance was 0.5 (EC50 in mg ml1) obtained from linear regression analysis.

***β*-Carotene bleaching test (BCBT)**

A modification of the method described by Koleva et al.[8] was employed. *β*-carotene (2 mg) was dissolved in 20 mL chloroform and to 4 mL of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40°C and 100 mL of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. Sample EOs and reference compounds (BHT and BHA) were prepared in ethanol. An aliquot (150 µL) of the *β*-carotene: linoleic acid emulsion was distributed in each of the wells of 96-well microtitre plates and methanolic solutions of the test samples (10 µL) were added. Three replicates were prepared for each of the samples. The microtitre plates were incubated at 50°C for 120 min, and the absorbance was measured using a model EAR 400 microtitre reader (Labsystems Multiskan MS) at 470 nm. Readings of all samples were performed immediately (t = 0 min) and after 120 min of incubation. The antioxidant activity (AA) of the extracts was evaluated in term of *β*-carotene blanching using the following formula:

AA (%) = [(A0-A1)/A0]*100 (2)

Where A0 is the absorbance of the control at 0 min, and A1 is the absorbance of the sample at 120 min. All samples were analyzed in three replications. The results are expressed as IC50 values ($\mu g.ml^{-1}$).

III. Result

Essential oil yield of aerial parts of *P. denticulatum*

The hydrodistillation of 100 g of the aerial parts of *P. denticulatum* gave pale oil with a fruity odor whose contents represent 0.07 ± 0.01% of the mass of the plant material initially used.

Antioxidant activity of essential oils of *P. denticulatum*

The antioxidant potentialities of the essential oils of *P. denticulatum* were evaluated by three different tests, namely the antiradical test against DPPH, the reducing power of iron and the bleaching inhibition capacity of *β*-carotene. All the results of these tests are shown in Table 1.

Examination of the results of the anti-free radical power showed that the essential oils of *P. denticulatum* exhibit an IC_{50} value equal to 8.3 $mg.mL^{-1}$, indicating a moderate activity. However, the reducing power of essential oils (EC_{50} = 1.8± 0.1 $mg.mL^{-1}$) is quite high. The results of the bleaching inhibitory activity of *β*-carotene expressed a fairly high IC_{50} value (IC_{50} = 4.9 $mg.mL^{-1}$) in relation to the nature of the test.

Table no 1: The *In vitro* antioxidant activities of *P. denticulatum* essential oils. DPPH test: 2,2-Diphenyl-1-picrylhydrazyl test; FRAP test: Ferric Reducing Antioxidant Power; BCBT: *β*-Carotene Bleaching Test; BHA: Butylated hydroxyanisole; BHT: butylated hydroxytoluene; AsA: Ascorbic Acid; CI_{50}: Inhibiting Concentration of 50% of the synthetic radical DPPH; CE_{50}: Effective Concentration at which the absorbance was 0.5.

		DPPH (IC_{50}: $mg.mL^{-1}$)	FRAP (EC_{50}:$mg.mL^{-1}$)	BCBT (IC_{50}:$mg.mL^{-1}$)
Essential oils		8.3 ±0.1	1.8±0.1	4.9±0.9
Positive controls	BHT	11.5±0.4	-	75±0.1
	BHA	6.1±0.3	-	48±0.2
	AsA	-	37.3±0.3	-

Identification of the chemical composition of essential oils from the aerial parts of *P. denticulatum* by CG/MS

The analysis of the chemical composition of the essential oils of *P. denticulatum* by gas chromatography coupled with mass spectroscopy revealed the chemical richness of this species. Indeed, these essential oils contained 54 constituents representing 93.53% of the totality of the oils identified. Hexyl butanoate (13.89%), Bicyclogermacrene (10.07%), and Nerolidol (11.47%) represented the main compounds (Table 2). In addition, other compounds were present in fairly large proportions such as Trans-2-hexenyl butyrate (4.2%), Alloaromadendrene (7.32%) and 5,9-Dimethyl-3-(1'-methylethyl)-1',10-epoxy-1,2,3,5,6,7,8,9-octahydronaphthalene (5.19%). The essential oil of *P. denticulatum* wes dominated by sesquiterpene hydrocarbons, representing almost one third (30.76%) of the total oil composition. Oxygenated sesquiterpenes came second with 20.63% and fatty acid esters just after with 18.86%, while phenylpropanoides were the least, representing only 4.48%.

Table no 2: Chemical composition of essential oils from the aerial parts of *P. denticulatum*. Components are listed in order of elution in apolar column (HP-5). RI^A, RI^B: retention indices calculated using respectively an apolar column (HP-5MS) and polar column (HP Innowax). Compound proportions were calculated from the chromatograms obtained on the HP Innowax column.

	Identified compounds	RI^A	RI^B	%
1	Sabinene	977	976	0.11±0.02
3	p-cymene	1026	1026	0.34±0.07
4	γ-terpinene	1062	1061	0.29±0.05
2	α-terpinolene	1083	1092	0.1±0.01
5	α-thujone	1102	1113	0.15±0.03
6	Terpinen-4-ol	1178	1178	0.14±0.04
7	Hexyl butanoate	1193	1191	13.9±1.1
8	trans-2-hexenyl butyrate	1195	1195	5.51±0.5
9	Vitispirane	1271	1242	0.73±0.07
11	Bicycloelemene	1328	1328	0.13±0.01
12	α-ylangene	1361	1372	0.37±0.03
45	Hydrocinnamic acid	1369	-	1.29±0.3
14	Hexyl caproate	1385	1385	0.17±0.07
15	Cyclooctyl methyl ether	-	-	3.24±0.3
16	γ-Dihydroionone	-	1396	0.15±0.03

18	Caryophyllene	1423	1604	3.17±0.3
19	γ-Maaliene	1435	-	0.11±0.01
20	3,7-Guaiadiene	1444	1810	1.29±0.1
23	Alloaromadendrene	1454	1661	10.53±0.8
22	α-Humulene	1456	1687	0.23±0.07
27	α-Amorphene	1466	1678	1.15±0.3
17	α-Gurjunene	1475	-	0.18±0.07
24	Germacrene D	1485	1726	0.43±0.13
25	Bicyclogermacrene	1493	1756	10.07±1.7
30	δ-Selinene	1496	-	0.96±0.05
39	Cubenene	1522	1799	1.16±0.07
26	Isodihydroagarofuran	-	-	5.29±0.7
28	δ-Cadinene	1528	1773	0.71±0.1
29	Selina-3,7(11)-diene	1540	-	0.36±0.07
31	α-Calacorene	1542	1914	0.15±0.03
33	Hydrocinnamyl isobutyrate	-	-	0.24±0.05
38	Ledol	1560	2030	2.17±0.6
34	Nerolidol	1565	2050	11.47±1.7
36	Spathulenol	1566	2150	2.69±0.7
37	Viridiflorol	1580	2098	1.66±0.8
35	Hexyl octanoate	1581	-	1.64±0.5
40	8-Epi-γ-eudesmol	-	-	2.06±0.5
41	Isospathulenol	1631	2223	0.35±0.9
42	τ-Muurolol	1648	2209	0.23±0.04
43	Caprylate	-	-	0.14±0.03
44	n-Undec heptyl -10-enylbenzene	-	-	0.79±0.1
46	3-Phenylpropyl 3-chloropropanoate	-	-	0.52±0.1
47	Ambrox	-	-	1.64±0.2
48	6,6-Dimethylhepta-2,4-diene	-	-	0.56±0.1
49	3-Methyl-3-phenylazetidine	-	-	0.14±0.05
50	Butyl cinnamate	-	-	3.34±0.2
51	Sclareoloxide	1906	-	1.23±0.2
53	Manoyl oxide	1989	2376	1.07±0.3
Total identified				93.53±5.46
Monoterpene Hydrocarbons				0.84±0.2
Oxygenated Monoterpenes				0.29±0.0.6
Sesquiterpene Hydrocarbons				30.76±3.2
Oxygenated Sesquiterpenes				20.63±1.9
Fatty acid esters				18.86±2.1
Phenylpropanoïds				4.48±0.7
Others				17.67±2.3

IV. Discussion

The results showed that essential oil yields of *P. denticulatum* aerial parts were equal to 0.07% of the dry matter, meaning approximately 0.7 $ml.Kg^{-1}$ DM. This yield is modest since essential oils are almost produced in small amounts. High levels such as that of the clove flower bud (150 $ml.Kg^{-1}$ and more in the dry matter) are exceptional[9]. The quantities thus determined are lower than the known standards in other species of the *Pelargonium* genus such as *P. graveolens* whose aerial parts yield in essential oils is around 0.15% of the dry matter[10].

In addition, the identification of the chemical composition of essential oils of *P. denticulatum* by GC-MS revealed the dominance of sesquiterpenes with both hydrocarbon and oxygenated forms, and to a lesser extent by fatty acid esters. The major compounds were Hexyl butanoate, Bicyclogermacrene, and Nerolidol. This sesquiterpene-rich chemical composition appears to be a common trait among related species of this genus[4]. However, the abundance of fatty acid esters seems to be an exclusive feature of this species. Indeed, the essential oil of *P. graveolens* is essentially made up of monoterpene alcohols and esters, in addition to sesquiterpene hydrocarbons[10]. A second study focused on the identification of the essential oils of 18 species of the genus *Pelargonium* and showed that the volatile composition of the latter is very variable but with the presence of good proportions of sesquiterpenes in the majority of the species studied[4].

The antioxidant potential of essential oils can be significantly related to different mechanisms such as free radical scavenging, metal ion chelation and peroxide decomposition[11], hence the need to use several tests to study it. The antioxidant potential of *P. denticulatum* is, to the best of our knowledge, explored for the first time in this study. The capacity of essential oils to act as antiradical molecules thus expressed was farly higher than

that determined in essential oils extracted from *Pelargonium graveolens*, whose chemical composition was dominated by oxygenated monoterpenes[12]. Besides, the reducing capacity of iron and the bleaching inhibitory activity of *β*-carotene were very interesting. Through these three tests, it appears that *P. denticulatum* has an essential oil with specific antioxidant potential, which can be explained by the chemical composition of the latter. Indeed, the biological activity of essential oils depends on the chemical composition and functional groups (alcohol, phenol, terpenes and ketones). Besides, the nature of terpene compounds and their proportions play a determining role in the antioxidant activity of EOs. This property, reported in several works, seems to be attributed to oxygenated monoterpenes, in particular phenols and aldehydes[13]. Nevertheless, these chemical classes are very weakly represented in the EOs of *P. denticulatum* which explains, in a way, their moderate antioxidant potential. Furthermore, it is well known that the presence of sesquiterpene hydrocarbons and minor terpenoids is attributed to low antioxidant activity[11].

The antioxidant activity thus determined by the essential oils of *P. denticulatum* can be linked to their richness on oxygenated sesquiterpene (20.63%). In fact, previous studies suggested that monoterpene hydrocarbons, monoterpenes and/or oxygenated sesquiterpenes represent the main antioxidant compounds in plants[3,11,13,14].

In addition to their antioxidant properties thus demonstrated, as well as their antimicrobial potentialities previously studied[4], the essential oils of *P. denticulatum* enjoy a very varied chemical composition and rich in metabolites with various interests, especially in food and pharmaceutical industries. Indeed, the bibliography has described the multiple uses, especially of the three main compounds of the essential oils of this species. Hexyl butanoate is the ester of butanoic acid and hexanol used as a flavoring in the food industry and perfumery. It has a strong fruity smell and is present in several fruits and berries. It is an important constituent of fruity flavors in food and cosmetic products[15]. Nerodiol has a woody aroma reminiscent of fresh bark. It is used as a food flavoring agent and in perfumery. In addition, this sesquiterpene alcohol enhances the penetration of therapeutic drugs percutaneously[16]. Bicyclogermacrene has many biological properties: antimicrobial, insecticide, fungicide and bactericide, as it is effective on ticks[17].

V. Conclusion

According to these different results thus obtained, it is appropriate to consider *P. denticulatum* as an important aromatic and medicinal species representing a valuable source of antioxidant molecules. Its qualitative and quantitative richness in these metabolites encourages its exploitation in industrial applications covering the pharmaceutical, cosmetic and food-processing fields.

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