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Essential oil composition and antioxidant activity of hydromethanolic extract from the flowers, leaves and stems of *Callistemon citrinus* (Curtis) Skeels

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Essential oils of flowers, leaves and stems of *Callistemon citrinus* (Curtis) Skeels (Family-Myrtaceae) were obtained by hydrodistillation and identified by GC and GC/MS. (46, 26, 46) components which accounted for over (94.5, 93.4, 94.1%) of flowers, leaves and stems oils were identified, respectively. The main components in the oils of plant parts were: 1, 8-cineol (16.1, 67.6, 41.3 %), α-pinene (25.7, 9.4, 19.1 %) and β-pinene (7.3, 1.3, 3.5 %), respectively. Essential oil of the leaves and hydromethanolic extract of flowers, leaves and stems of *C. citrinus* were emphasized as antioxidant and free radical scavenger. The samples were measured in terms of hydrogen donating or free radical scavenging ability. Results indicated the best anti-oxidant activity for flowers extract.

Keywords: Essential oil, *Callistemon citrinus*, 1, 8-Cineole, Antioxidant activity.

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Introduction

Family Myrtaceae consists of 3800 species, which are distributed in 140 genera. The plants are mostly found in tropical and subtropical regions of the world. The genus *Callistemon* R. Br. (commonly known as bottlebrush), belongs to this family Myrtaceae, which contain approximately 34 species. *Callistemon* species have attractive narrow foliage and white papery bark. The leaves are aromatic and lanceolate, mostly 40-70 mm long and 3-6 mm wide. The flowers are brush like, resembling a traditional bottlebrush. Flowering is normally in spring and early summer, but conditions may cause flowering at other times of the year[1].

The genus *Callistemon* R.Br. is known in folk medicine for its anti-cough, anti-bronchitis and insecticidal effects. Its volatile oils have been utilized as antimicrobial and antifungal agents[2-4]. Diverse bioactivity studies of different species of the genus have been reported. Essential oils of *C. citrinus* (Curtis) Skeels*, C. viminalis* (Soland.) Cheel and *C. lanceolatus* (Sm.) Sweet from Australia, Egypt, India, Pakistan and Reunion Island, have been previously reported. 1,8-Cineole was the predominant constituent of the oils. Other significant components included: α-pinene, β-pinene, myrcene, limonene, linalool and menthyl acetate[5-9]. Moreover, anthelmintic, anti-inflammatory, antibacterial, antioxidant and cardioprotective activity of some species in the genus *Callistemon* have been documented[10-13].

C. citrinus, which is a pollution tolerant plant and has a great medicinal value. Traditional uses of the aerial parts of this species in ethnic tribal communities are in practice and very little is known about its importance on scientific grounds. Biological activity of the essential oils and extracts of its leaves such as Calcium channel blocking activity of fruits[14], acute toxicity and cytotoxic activities of the crude methanol extract of fruits[15], inhibitory effects on the acetylcholine induced contraction[12], antifungal[16], antibacterial[17-19] and anthelmintic[20] activity of the plant have also been studied.

In this study, we analyzed *C. citrinus* which is cultivated in Iran as an ornamental plant, for antioxidant activity. Scientists have always considered antioxidant activity of natural products. Free radicals are active molecules that are present everywhere and may be of internal or external origin. One of the most important destructive effects of free radicals is promoting lipid peroxidation. This process causes the destruction of membrane and changes

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activity of intra membrane enzymes and other proteins and releases radicals that are initially harmful to tissue cells. To the best of our knowledge, this is the first time that essential oil of leaves and 80 % methanolic extract of flowers, leaves and stems of *C. citrinus* have been emphasized as antioxidant and free radical scavenger. Essential oil composition of separate parts of the plant such as flowers, leaves and stems of *C. citrinus* are also investigated in this paper.

Materials and Methods

Plant material

The aerial part of *C. citrinus* was collected from a cultivated plant in the seaside area of Roodsar (Province of Gilan), Iran, in June 2012. Identification of the plant was done by using the identification key in the Manual of Cultivated Trees and Shrubs[21].

Isolation of the volatile oils

The aerial parts of the plant (flowers, leaves and stems) were dried in the dark, then 77, 100, 116 g of the plant parts were separately hydro distilled using a Clevenger type apparatus for 3h. Finally the oils were dried over anhydrous sulfate.

Preparation of the extracts

The extracts were prepared by soaking separate dried and powdered plant parts in methanol 80 % (1g/6mL) for one week. Occasional shaking and stirring was done. Then filtrated by Whatman paper No.4 and stored at 4°C prior to further use.

Gas Chromatography (GC)

GC analysis was performed on a Shimadzu GC-15A equipped with a split/split-less injector (250ºC). N_2 was used as carrier gas (1 mL/min). The DB-5 (50 m ×0.2 mm, film thickness 0.32µm) capillary column was used. The column temperature was kept at 60ºC for 3 min. and then heated to 220ºC with a 5ºC/min rate. Relative percentage amount of the constituents, calculated from peak area using a Shimadzu C-R4A Chromatopac without using of correction factors.

Gas Chromatography-Mass Spectroscopy (GC/MS)

GC-MS analysis was performed using a mass selective detector (Agilent 5973) coupled with a gas chromatograph (Agilent 6890), equipped with a capillary column (HP-5MS) (30 × 0.25 mm, film thickness 0.25 µM). The column temperature was kept at 50ºC for 5 min. and programmed to 240ºC at a rate of 3ºC/min.Then the temperature was involved to 300ºC at the rate of 15ºC /min. and was kept constant at 300 ºC for 3 min. The injector temperature was 290ºC and the flow rate of Helium as carrier gas was 0.8 mL/min. The MS operating parameters were as follows: Ionization energy 70 ev, ion source temperature 220ºC.

Identification of constituents

The constituents of each oil was identified by comparing their retention indices (RI) relative to n-alkanes, computer matching with the Wiley library and confirmed by comparing their Mass spectra with those of authentic samples or with data already available in the literature[22]. The percentage composition of the identified compounds was computed from the GC peak area without any correction factor.

Antioxidant activity

Antioxidant activity of separate aerial parts of *C. citrinus* were evaluated by comparing with standard Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (sigma-Aldrich, USA) on the basis of scavenging activity of the stable DPPH (1, 1-diphenyl-2-picryl hydrazyl radical) (Sigma-Aldrich, USA) free radical. 100 mL of DPPH solution (40 µg/mL) was prepared by dissolving 4 mg of DPPH in methanol (Merck, Germany) in a 100 mL volumetric flask. 5 mL of Trolox solution (0.3 mg/mL) was also prepared by dissolving 1.5 mg of Trolox in methanol. 2.5 mL of DPPH solution (freshly prepared) was added to 10 µL of essential oil and each extracts solutions separately. After 30 min incubation in dark, absorbance of each test tube was taken by a spectrophotometer (Unico UV-2100, China) at 517 nm and the percentage of scavenged DPPH was calculated using the following equation: DPPH scavenging activity=$100[(A_c-A_s)/A_c)]$, where A_c is the absorbance of control (distilled water) and A_s is the absorbance of sample. The assays were carried out in duplicate and average values were considered for the percentage inhibition capacity[23].

Results

The yield of oils isolated from flowers, leaves and stems of *C. citrinus* was (0.13, 0.10 and 0.08%) (v/w), respectively. The 46, 26, 46 components which accounted for over 94.5, 93.4 and 94.1% of flowers, leaves and stems oils were identified, respectively. The identified compounds are listed in Table 1 in which the percentage and Retention Indices are also given. The oils were found to be rich in monoterpens (88.2, 91.8, 82.9 %) in all three parts of the plant. The

Table 1—Essential oil composition of flowers, leaves and stems of *Callistemon citrinus*(Curtis) Skeels

Compounds	RI[a]	Flowers	Leaves	Stems
α-Pinene	939	25.7	9.4	19.1
Camphene	953	-	-	0.1
β-Pinene	980	7.3	1.3	3.5
myrcene	991	-	0.1	1.0
p-Mentha-1,8-diene	1004	-	0.2	-
δ-3-Carene	1011	3.6	-	5.4
1,8-Cineole	1033	16.1	67.6	41.3
E-β-Ocimene	1050	-	0.5	-
γ-Terpinene	1062	-	-	2.2
Octanol	1070	-	-	0.1
Terpinolene	1088	1.2	0.1	0.6
Linalool	1098	5.3	0.4	1.7
Endo-Fenchol	1112	0.3	0.1	0.1
Terpin-1-ol	1134	0.2	-	0.1
trans-Pinocarveol	1139	trace	trace	0.3
trans-β-Dihydro terpineol	1158	0.2	-	-
Pinocarvone	1162	-	0.1	0.1
Borneol	1165	0.4	0.3	0.2
Terpin-4-ol	1177	7.0	1.4	1.7
α-Terpineol	1189	18.1	4.7	4.1
Trans-Carveole	1217	-	0.2	0.1
Nerol	1228	0.1	-	0.1
Carveol methyl ether	1244	-	0.1	-
Geraniol	1255	0.4	-	0.1
Geranial	1270	0.1	-	-
Bornyl acetate	1285	0.1	-	-
Thymol	1290	-	3.7	-
Carvacrol	1298	0.1	0.3	-
Methyl geranate	1323	1.7	1.3	1.1
Neo-iso-Verbenol acetate	1328	0.1	-	trace
Neryl acetate	1365	0.1	-	trace
α-Copaene	1376	0.1	-	trace
Geranyl acetate	1383	0.1	-	trace
α-Gurjunene	1409	trace	-	-
β-Caryophyllene	1418	0.2	0.1	0.9
Aromadendrene	1439	0.1	0.2	0.2
Geranyl acetone	1453	-	-	trace
α-Humulene	1454	0.1	-	0.1
Allo-aromadendrene	1461	0.1	0.1	0.2
γ-Gurjunene	1473	0.1	-	0.1
Viridiflorene	1493	0.2	-	0.3
δ-Cadinene	1524	-	-	0.1
epi-Longipinanol	1561	0.1	0.1	-
Ledol	1565	0.3	0.2	0.5
Spathulenol	1576	0.3	0.1	2.7
Glubolol	1583	2.0	0.8	2.4
cis-β-Elemenone	1594	1.1	-	1.5
iso-Spathulenol	1631	0.6	-	0.8
α-Eudesmol	1652	0.1	-	0.1
epi-α-Bisabolol	1686	trace	-	-
Caryophyllene acetate	1700	0.4	-	0.1
E,E-Farnesol	1722	trace	-	0.1
Tetradecanoic acid	1765	0.1	-	0.1
Octadecane	1793	trace	-	trace
Dibuthyl phtalate	1959	0.2	-	0.2
Hexadecanoic acid	1962	0.2	-	0.6
Eicosane	1994	trace	-	-
(Z)-9-Octadecenoic acid	2141	trace	-	0.1
Total		94.5	93.4	94.1

RI [a] = Retention Index, determined with reference to a homologous series of normal alkanes on HP-5MS column, trace ≤ 0.05%

Table 2—Inhibition capacity of flowers, leaves and stems of Callistemon citrinus(Curtis) Skeels

Samples	Absorbance[a]	Inhibition[b]
A	0.087	93.32
B	0.468	64.11
C	0.728	44.17
D	1.192	8.58
E	1.127	13.57
F	1.304	0

Extracts of A: Flowers; B. Leaves; C. Stems; D. Leaves essential oil; E. Trolox as standard; F. Distilled water as blank. a. Absorbance of the samples measured in 517 nm; b. Inhibition of free radical DPPH in each solution, calculated in percentage

most abundant constituents were oxygenated monoterpenes (50.4, 80.2, 51%). 1, 8-Cineol (16.1, 67.6, 41.3%) constituting the bulk of the oils of leaves and stems. Monoterpene hydrocarbons (37.8, 11.6, 31.9%) including α-pinene (25.7, 9.4, 19.1%) and β-pinene (7.3, 1.3, 3.5%) were found more in flowers. Furthermore α-terpineol (18.1, 4.7, 4.1%), terpin-4-ol (7.0, 1.4, 1.7%) and linalool (5.3, 0.4, 1.7%) were the other oxygenated compounds with high amount in flowers. The oils of flower and stem (3.6, 5.4%) also contain δ-3-carene and thymol in the oil of leaves (3.7%). Sesquiterpenes were seen (5.8, 1.6, 10.1%), respectively in total oils.

The measured antioxidant activity of the essential oil and extracts of *C. citrinus* is listed in Table 2. The highest antioxidant activity was observed for flowers extract, showing much better result than the scavenging effect of the standard antioxidant, Trolox. The radical scavenging values were also good for leaves and stems, while essential oil showed the less antioxidant activity.

Discussion

Although the essential oil composition from the leaves of *C. citrinus* in different countries has been studied[17, 19, 24], the flowers and stems have not been mentioned. There are distinctions in the yield and constituents of the oils, which could be attributed to differences in generic and geographical/environmental conditions. The abundance of 1, 8-cineole in the essential oils of our samples makes them similar to those obtained in all previous studies, but a key difference in the oils lies in the relative quantities of α-pinene, β-pinene, limonene, linalool and α-terpineol.

Previous investigations on *Callistemon* species, showed antioxidant activity for the leaves extract of *C. lanceolatus* (Sm.) Sweet[25]. Essential oil obtained

by hydro distillation of the leaves and also leaves extract of *Callistemon comboynensis* Cheel showed high antioxidant activity[26]. One of the assays which is used for antioxidant activity is based on the ability of DPPH, to decolorize in the presence of anti oxidants. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The odd electron in DPPH radical is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, will be delocalized which can be quantitatively measured from the changes in absorbance[27].

In this study, we demonstrated that the 80% methanolic extracts of different parts of *C. citrinus*, especially the flowers are highly active antioxidant materials. It is extremely important to point out that antioxidant activity of plant material is well correlated with the content of phenolic compounds. So, the low antioxidant activity for essential oil of the leaves of *C. citrinus* can be explained because phenolic compounds were poorly found in the oil. Also, potent antioxidant activity of hydro methanolic extracts of flowers, leaves and stems can be a result of high amount of flavonoids and phenolic compounds.

Conclusion

The flowers of *C. citrinus* have a high antioxidant activity that can be used to treat several diseases in which there is an increase in free radical production. Additional *in vitro* antioxidant assays are needed to confirm the potential use of the species in disease treatment. *C. citrinus* is an abundant plant, so it can be mentioned as a valuable source of 1,8-cineole (eucalyptol) which has a range of antimicrobial, pesticidal and herbicidal activities[28-31]. The different plant parts can also be the subject of more studies on biological activities.

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