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Chemical composition and antioxidant activities of essential oil from Somsa (*Citrus aurantium* L.) in Phitsanulok province, Thailand

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Abstract

Citrus aurantium L. has been used as a traditional medicine worldwide for a very long time. Different parts of this plant offer valuable benefits to mankind. This study was carried out to investigate the chemical composition of essential oil from the leaves and peel of Somsa (*C. aurantium* L.) sampled from Phitsanulok Province, Northern Thailand and to evaluate their antioxidant activity against the free radical ABTS (2,2–Azino–Bis–3 Ethylbenzthiazoline– 6 Sulphonic Acid) and DPPH (2,2 Diphenyl–1– Picrylhydrazyl). Essential oil was extracted from the leaves and peel by hydro–distillation. Chemical composition of the extracted oil was then analyzed using Gas Chromatography Mass Spectrometry (GC-MS) and MS inert mass selective detector for characterization of the essential oils. Antioxidant activity of oil extracts from *C. aurantium* L. leaves and peel was analyzed by ABTS and DPPH assays. Twenty eight (28) different compounds were identified in essential oil from leaves representing 99.03% and peel representing 93.61% of total oil content. The main components of the oil extracted from leaves include 1,8 Cineole (38.45%), Sabinene (15.88%), Linalool L (11.47%), α - terpineol (8.63%) and Ocimene (6.88%). On the other hand, beta– myrcene (21.7%), Octanal (9.63%), α - pinene (8.42%), Germacrene – D (6.92%) and α - terpineol (6.31%) were identified as the major components in the peel oil. Antioxidant activity (inhibitory percentage) of essential oil from the leaves in DPPH assay (38.28%) and ABTS assay (18.58%) was higher compared to that of the peel oil (16.09% and 11.84%, respectively). Essential oils from both parts (leaves and peel) were more effective in DPPH assay than the ABTS assay.

Keywords: Somsa, *Citrus aurantium* L., Essential oil, Antioxidant, Phitsanulok.

1. Introduction

Citrus fruits (Rutaceae) are important fruits in the world that provide functional compounds such as folic acid, vitamin C, fibers and essential oils. The genus *citrus* of the family Rutaceae include several important fruits such as mandarins, oranges, limes, grapefruits and lemons. More than 80 million tons of *Citrus* fruits are produced annually worldwide, making them one of the most important horticultural crops in the world [1-3]. In Thailand, *Citrus* production takes place on a wide range of soils. An estimated amount of 100,145 tons *Citrus* fruits were produced in Thailand in 2015. Most of the *Citrus* fruits produced in Thailand are consumed domestically. Waste products produced during the processing of *Citrus* fruits are also economically valuable. Local communities in Phitsanulok, Thailand apply leave extracts as traditional medicine for treating skin diseases. It is also used as balsam [4-6] *C. aurantium* is widely distributed in Mediterranean countries. Chemical composition of essential oil extracted from leaves and peel of *C. aurantium* sampled from Constantine (Eastern

Algerian) has been analysed and compared to that of varieties grown in different parts of the world [1]. Azadi et al. [2] characterized the chemicals in leaf essential oil from *C. aurantium* L. cultivated in the north of Iran. The major compound in the oil was found to be linalool (39.4%), while peel essential oil mainly contained limonene (91.3%). However, leaf and peel essential oil in *C. aurantium* collected from Constantine contained mainly 18.6% and 12.0% linalool respectively. The composition and concentration of essential oil extracted from the same part(s) of *C. aurantium* growing in different parts of the world were found to be different. Essential oils are complex phytochemicals obtained from by-products of the citrus processing industry. Natural compounds found in essential oil are very important in health science [7-8]. Citrus essential oils are very important in medicine and are attracting particular interest from the pharmaceutical and cosmetics industries [9-10]. As a flavouring agent, essential oil is used in pharmaceutical industries to mask unpleasant tastes of drugs. *Citrus* essential oils are the most widely used essential oils in the food industry worldwide because they are safe [5,7]. They are extensively used as flavouring agents in a number of food products, including soft drinks, candies, dairy products and other sweet flavoring agents. Essential oils can also be used in confectionery products and in fragrance application [11-12]. Coumarins, flavonoids, vitamins and triterpenes found in essential oil from bitter orange, are widely used in the food and cosmetic industries [13]. Essential oil from citrus has also been reported to possess antifungal, antimicrobial, and radical scavenging activities (antioxidant) [5, 14-17]. This study is therefore aimed at analyzing the chemical composition of leaf and peel essential oils of *C. aurantium* L. collected from Phitsanulok province and to evaluate their antioxidant activity.

2. Methodology

2.1 Sample Preparation and Essential oil Extraction

The leaves and peel of Somsa (*C. aurantium* L.) were collected from Phitsanulok Province, Thailand. The fruits were washed and peeled carefully with a sharp knife to avoid damage of the oil glands. The leaves and peel were then dried at ambient temperature for a week. Essential oil was extracted from 100 g of leaves and peel of *C. aurantium* L. using hydro-distillation for 3.5 hours with a Clevenger apparatus. The extraction was continued at 100 ºC until there was no essential oil in the samples. Distillates of essential oil were dried with anhydrous sodium sulfate. The extracts were wrapped with aluminum foil (dark condition) and stored at 4 ºC.

2.2 GC – MS Analysis and Identification of Compounds

Essential oils were analysed by an Agilent 6890 GC-MS system equipped with a DB-5 capillary column (30 m length x 0.25 mm diameter, 0.25 µm film thickness) and an Agilent 5973 inert mass selective detector. The temperature of the column was set from 70 to 220 °C. The column temperature was held at 160 °C for 10 min, before raising it up to 220 °C at 5 °C/min and maintaining it for 2 minutes. Nitrogen was used as the carrier gas at a flow rate of 1 ml/min. A sample of 1.0 µl was injected using split mode (split ratio, 1:100). Essential oil components were identified and reported based on their relative percentage of peak area (determined with reference to a homologous series of normal (n) - alkanes). The result was compared with their mass spectral fragmentation patterns as reported in the literature, Wiley library for the chemical components.

2.3 Antioxidant Activity

2.3.1 DPPH assay

A 0.1 mM DPPH in absolute ethanol was used to determine the free radical scavenging activity following the method described by Rathee et al. [13] with slight modifications. Two µL of undiluted samples was directly reacted with 200 µL of 0.1 mM DPPH solution and transferred into a micro plate, BioTek Synergy HT. It was then homogenized and allowed to stay at room temperature for 25 minutes, avoiding light intensity. Absorbance was measured at 517 nm for reaction of DPPH with sample extract. Ethanol and DPPH free radical were used as blank and control, respectively.

2.3.2 ABTS assay

The antioxidant activity in ABTS assay was determined by following the method described by Thaipong et al. [16] with some modifications. Stock solutions of 3.5 mM ABTS in 10 ml distilled water (18.2 MΩ) and 70 mM potassium persulfate ($K_2S_2O_8$) in 1 ml distilled (18.2 MΩ) were prepared. A working solution comprising of a mixture of 5 ml 3.5 mM ABTS solution and 88 µl of 70 mM $K_2S_2O_8$ solution was then prepared by mixing the two solutions in the dark at room temperature and allowing them to react for 12 h. The solution was then diluted with distilled water to obtain a standard absorbance of 0.7±0.02 at 734 nm with the spectrophotometer.

Two µL of undiluted sample was directly reacted with 200 µL of the working solution of ABTS and transferred into a micro plate, BioTek Synergy HT. It was homogenized and allowed to stay at room temperature for 5 minutes, devoid of light intensity. Absorbance of ABTS reaction with samples extracts was measured at 734 nm. Distilled water and ABTS free radical were used as blank and control, respectively.

The effect of DPPH and ABTS antioxidant activity was calculated as inhibitory percentage of DPPH and ABTS discoloration by the following equation:

$$\text{Inhibition (\%)} = \frac{(Abs\ Control - Abs\ Sample)}{Abs\ Control} \times 100 \quad (1)$$

The result was expressed as inhibitory percentage (I %) of each sample (three replicates).

2.4 Experimental Design

Completely Randomized Design (CRD) was chosen for experimental design. One Way ANOVA was used to test for significant difference. The results were presented as mean ± standard deviation of three replications of antioxidant activities. The statistical analysis software version 16.0 from SPSS (Statistical Packages for Social Sciences) was used in this study.

3. Results and Discussion

3.1 Leaves and Peel Essential oil content and Their Composition

Essential oil from leaves and peel of *C. aurantium* L. was extracted by hydro-distillation process from 100 g of both parts (leaves and peel) at 100 ºC for 3.5 hours. Analysis of oil extracts from leaves (Figure 1) and peel (Figure 2) revealed twenty eight (28) different chemical compositions (Table 1).

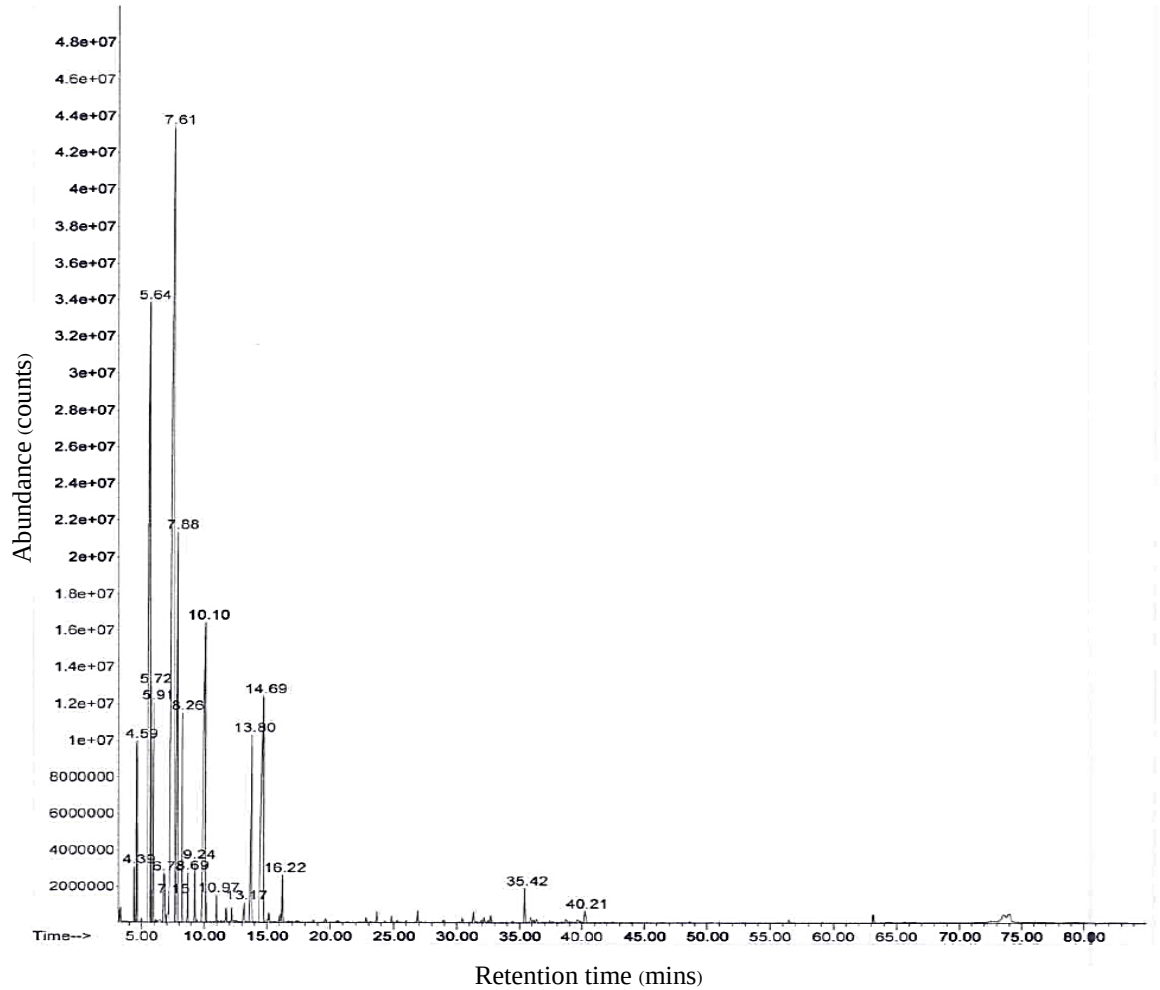

Figure 1 Chromatogram of leaf essential oil of *C. aurantium* L

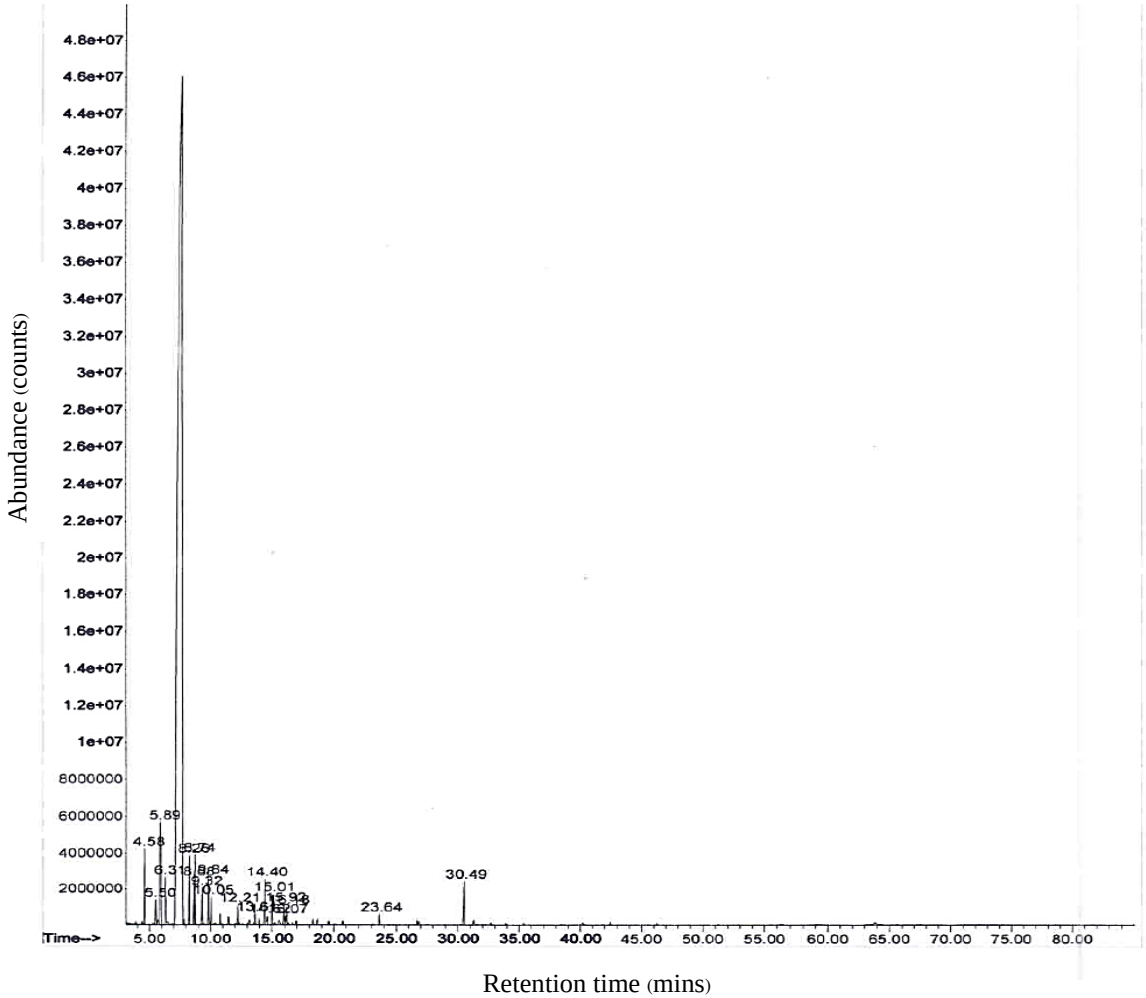

Figure 2 Chromatogram of peel essential oil of *C. aurantium* L

The peel of Somsa (*C. aurantium* L.) contains 2.45% (v/w) essential oil with a strong pleasant odour and clear yellow colour. The leaves on the other hand contain 0.18% (v/w) essential oil with clear appearance, fresh and neroli odour. A total of 4 ml and 0.4 ml essential oils were produced from 100 g of peel and leaves of *C. aurantium* L., respectively. Eighteen components each were identified in essential oil from leaves and peel representing 99.03% and 93.61% of total oil contents, respectively. Essential oil extracted from leaves contains the highest contents of 1,8 Cineole or eucalyptol (38.45%) but the lowest contents of dl – Limonene (0.21%). Meanwhile, peel essential oil contains the highest contents of β – Myrcene (21.7%) and the lowest contents of β – Citronellol (1.09%) compared to other components (Table 1). Similar components were also identified in both leaves and peel at different concentrations (Table 1). While oil from leaves contains α - Pinene (2.06%), Sabinene (15.88%), β – Myrcene (2.22%), γ – Terpinene (2.29%), Linalool L (11.47%) and α - Terpineol (8.63%), oil from peel was also made up of α - Pinene (8.42%), Sabinene (3.77%), β – Myrcene (21.7%), γ – Terpinene (5.51%), Linalool L (5%) and α - Terpineol (6.31%). The contents of Sabinene, Linalool L and α - terpineol in leaves oil were higher than those of oil from peel. However, α - Pinene, β – Myrcene and γ – Terpinene contents in oil from leaves were lower compared to those of peel oil (Table 1).

Table 1 Chemical composition of leaves and peel essential oils of *C. aurantium* L.

No.	Compounds	LEO **(%)**	PEO **(%)**
1	α - Thujene	0.64	-
2	α - Pinene	2.06	8.42
3	Sabinene	15.88	3.77
4	2 - β – Pinene	1.56	-
5	β – Myrcene	2.22	21.7
6	α - Terpinene	1.32	-
7	dl – Limonene	0.21	-
8	1,8 – Cineole	38.45	-
9	Ocimene	6.88	-
10	Octanal	-	9.63
11	γ – Terpinene	2.29	5.51
12	α - Terpinolene	0.68	-
13	Linalool Oxide CIS	-	5.63
14	Linalool Oxide (2)	-	3.83
15	Linalool L	11.47	5
16	Trans-p-menth-2-en-1-ol	0.3	-
17	Nonanal	-	2.58
18	Citronella	-	2.09
19	Terpinen - 4 - ol	4.84	1.25
20	α - Terpineol	8.63	6.31
21	Decanal	-	3.7
22	Nerol	-	2.66
23	β – Citronellol	-	1.09
24	Thymol methyl ether	0.64	2.1
25	Nerolidol	0.59	-
26	Neryl Acetate	-	1.42
27	β – Eudesmol	0.37	-
28	Germacrene – D	-	6.92

Note: Components are arranged in order of elution from DB–5 Column and reported as percentage of peak area.

The major components of essential oil (>6%) extracted from the leaves include 1,8 cineole (38.45%), sabinene (15.88%), linalool L (11.47%), α - terpineol (8.63%) and ocimene (6.88%). On the other hand, β – myrcene (21.7%), octanal (9.63%), α - pinene (8.42%), germacrene – D (6.92%) and α - terpineol (6.31%) were the main components in oil extracted from peel. The major components of essential oil from this study were more than those oils extracted from *C. aurantium* growing in other parts of the world as well as other species such as *C. aurantifolia* and *C. hystrix* (Table 2 and 3).

Sabinene (15.88%) and 1,8 Cineole (38.45%) contents in leaves essential oil from this study were higher (Table 2) than those of oil extracted from the leaves of *C. aurantium* in northern Iran (0,5% and 0%, respectively), Constantine, Algeria (1.8% and 3%, respectively) and Greece (0.37% and 0%, respectively) [1,2,14]

The concentrations of the main components in peel essential oil from the current study were also higher than the ones identified in peel oil of *C. aurantium* from north Iran [2], Tunisia [18], Greece [14] and other species like *C. aurantifolia* [11] and *C. hystrix* [9] (Table 3). All of the chemical compositions of peel oil from this study were also identified in oil extracted from *C. aurantium* peel in Tunisia [18], North Iran [2] and Greece [14]. Contents of essential oils from leaves and peels in this study were again higher than oil extracted from the leaves and peels of *C. aurantifolia* [11] and *C. hystrix* [19].

Table 2 Comparison of major components of leaves essential oil from this study with other studies

Species	location	Sabinene (%)	1.8 Cineole (%)	Linalool (%)	α-Terpineol (%)	Ocimene (%)	Reference
C. aurantium	North Iran	0.50	n.d.	n.d.	7.20	n.d.	[2]
C. aurantium	Algeria	1.80	3.00	18.6	15.10	n.d.	[1]
C. aurantium	Greece	0.40	n.d.	58.21 (YP) 36.03 (OP)	36.03 (YP) 12.89 (OP)	n.d.	[14]
C. hystrix	Paris)	n.d.	6.40	2.80	7.80	n.d.	[9]
C. aurantifolia	Italy	0.50	n.d.	10.60	n.d.	n.d.	[11]
C. aurantium	Thailand	15.88	38.45	11.47	8.63	6.88	This study

Note: n.d., not detected; YP, young plant; OP, old plant

Table 3 Comparison major components of peel essential oil from this study with other study

Species	location	α-pinene (%)	Myrcene (%)	Octanal (%)	α-Terpineol (%)	Germacrene (%)	Reference
C. aurantium	North Iran	0.10	3.00	0.20	0.20	n.d.	[2]
C. aurantium	Tunisia	0.56	1.63	0.38	0.93	n.d.	[18]
C. aurantium	Greece	0.52	2.00	0.24	0.13	n.d.	[14]
C. hystrix	Paris	3.23	n.d.	n.d.	8.35	n.d.	[9]
C. aurantifolia	Italy	2.01	n.d.	n.d.	0.40	n.d.	[11]
C. aurantium	Thailand	8.42	21.70	9.63	6.31	6.92	This study

Note: n.d., not detected

3.2 Antioxidant Activity

Essential oils from both leaves and peel were reacted with the free radicals ABTS and DPPH. Table 4 shows the inhibitory percentage of both leaves and peel oils on ABTS and DPPH.

Table 4 Antioxidant activities of leaves and peel essential oils on ABTS and DPPH

	Antioxidant Activity **(%)**	
Sample	ABTS	DPPH
Leaves essential oil	18.58 ± 0.8^{b}	38.28 ± 1.4^{a}
Peel essential oil	11.84 ± 0.9^{d}	16.09 ± 0.7^{c}

Note: Values are means ± standard deviation. Values with different letters in rows or columns are significantly different ($P < 0.05$)

Antioxidant activities of essential oils from leaves and peel in DPPH assay were 38.28% and 16.09%, respectively, while those in ABTS assay were 18.58% and 11.84%, respectively. Antioxidant activity of leaves oil was higher than peel oil in both assays. Essential oils from leaves were more effective in scavenging ABTS and DPPH radicals compared to that of peel. Bioactive compounds such as γ-terpinene, terpinolene, geraniol, β-pinene and myrcene, even at low concentrations, had high antioxidant activities [14].

Antioxidant activities of essential oil from both leaves and peel in the current study were compared with other studies by the ability of the oil to act on ABTS and DPPH radicals (Table 5). The results showed that peel essential oil in this study had stronger activity in DPPH assay compared to that of essential oil from peel of *C. aurantium* in Eastern Morocco [3]. Antioxidant activity of essential oils extracted from old leaves (94.36%) of *C. aurantium* on DPPH free radical studied in Greece [14], was higher than those of extracted oils from peels (16.09%) and leaves (38.28%) in the current study. On the other hand, the activity of leaves extracted oils in this study was higher than that of essential oils from young leaves (22.79%) and peels (19.29%) of *C. aurantium* in the study done in Greece [14]. Oil extracts of peel and leaves from *C. aurantifolia* evaluated in Italy [11], had stronger radical scavenging activity in both DPPH and ABTS assays than those of leaves and peel in this study. The antioxidant activities of oil from both plant parts in both DPPH and ABTS assay in the current study were again lower than those of oils from leaves and peel of *C. aurantium* in Greece (Table 5).

Table 5 Comparison antioxidant activities of peel and leaves essential oil from this study with other study

		Leaves (%)		Peel (%)		
Species	location	DPPH	ABTS	DPPH	ABTS	Reference
C. aurantium	Morocco	n.d.	n.d.	12.38	n.d.	[3]
C. aurantium	Greece	22.79 (YP) 94.36 (OP)	n.d.	19.29	n.d.	[14]
C. aurantifolia	Italy	76.90	21.90	78.30	18.70	[11]
C. aurantium	Thailand	38.28	18.58	16.09	11.84	This study

Note: n.d., not detected; YP, young plant; OP, old plant

4. Conclusion

Yield of essential oil from the peel of *C. aurantium* L. was greater than that of leaves by using hydro-distillation method. Twenty eight different chemical compositions were found in both leaves and peel essential oils. The major compounds of leaves essential oil were 1,8 cineole or eucalyptol, sabinene, α - terpineol, ocimene, and linalool, whereas myrcene, octanal, α - pinene, α - terpineol and germacrene were the main compounds found in peel oil. Similar components were also identified in both leaves and peel at different concentrations. Leaves essential oil showed significantly greater antioxidant activity than that of essential oil from peel in DPPH and ABTS assays.

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