

Full Length Research Paper

***Citrus reticulata* Blanco cv. Santra leaf and fruit peel: A common waste products, volatile oils composition and biological activities**

Dalia I. Hamdan¹, Maged E. Mohamed^{1,2} and Assem M. El-Shazly^{1*}

¹Department of Pharmacognosy, Faculty of Pharmacy, University of Zagazig, Zagazig 44519, Egypt.

²Department of Pharmaceutical Sciences, School of Clinical Pharmacy, University of King Faisal, P.P. 380, Ahsaa 31982, Kingdom of Saudi Arabia.

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The components of essential oils from the leaf and fruit peel of *Citrus reticulata* Blanco cv. Santra (santra mandarin) cultivated in Egypt were explored qualitatively and quantitatively using GLC and GLC/MS and 131 components were identified and quantified. In the leaf oil, one hundred and nine (109) compounds were determined with sabinene (23.10%) and linalool (21.20%) as a major component. The total identified components in the fruit peel oil were sixty-four and limonene (79.64%) was the most abundant. Santra mandarin volatile components showed good anti-inflammatory activity represented by its effect on tumor necrosis factor- α and nitric oxide. Egyptian santra mandarin chemotype was distinguished as limonene for peel oil while sabinene/linalool was observed for leaf oil. This study could be the milestone for the reuse and recycling of Egyptian santra mandarin leaves and fruit peel as a common waste products. The study also suggests the use of these wastes for the production of more valuable pure compounds such as limonene, sabinene and linalool.

Key words: Santra mandarin, essential oils, gas chromatography (GLC) and GLC/MS, chemotaxonomy, anti-inflammatory, antioxidant activities.

INTRODUCTION

Citrus plants are well-known crops all over the world with potential socio-economic influence. They are well-known for their flavor, nutritional value and medicinal features. The medicinal activities for this genus are attributed to the presence of many medicinally active secondary metabolites such as essential oils (Caccioni et al., 1998;

Lota et al., 2000, 2001; Dugo and Di Giacomo, 2002; Sutthanont et al., 2010; Espina et al., 2010), flavonoids (Tripoli et al., 2007; Abeysinghe et al., 2007; Du and Chen, 2010), limonoids (Rouseff and Nagy, 1982; Jayaprakasha et al., 1997), furanocoumarins, sterols, carotenoids and alkaloids (Ladaniya, 2008; He et al.,

*Corresponding author. E-mail: assemels2002@yahoo.co.uk. Tel: +2(0)552303266. Fax: +2(0)552303266.

2010). Many *Citrus* species among the different variety of *Citrus reticulata*, are acknowledged as food supplements and nutraceuticals for many physiological, pharmacological and medicinal activities such as antimicrobial (Chutia et al., 2009; Espina et al., 2010; Singh et al., 2010; Sultana et al., 2012; Tao et al., 2014), antioxidant (Goulas and Manganaris, 2011; Barros et al., 2012; Zhang et al., 2014), anti-inflammatory (Menichini et al., 2011), anticancer (Manthey and Guthrie, 2002; Benavente-Garcia and Castillo, 2008), antiproliferative (Du and Chen, 2010), anti-pulmonary fibrosis (Zhou et al., 2013), hypoglycemic (Aruoma et al., 2012) and insecticidal (Jayaprakasha et al., 1997; Sutthanont et al., 2010) activities.

Different varieties of *C. reticulata* exhibit a great diversity in morphological, horticultural characters and secondary metabolite constituents. These plants were also well known for many folk medicine uses such as fever, snakebite, stomachache, edema, cardiac diseases, bronchitis and asthma (Yabesh et al., 2014).

The chemical constituents of peel and leaf essential oils of 15 species of mandarins among 41 varieties of *C. reticulata* were investigated (Lota et al., 2000, 2001). The mandarin peel essential oil was reported to have two major chemotypes, limonene and limonene/ γ -terpinene. The leaf oil showed variation in components and distinguished for peel oils with three major chemotypes: sabinene/linalool, linalool/ γ -terpinene and methyl N-methylanthranilate (Lota et al., 2000). Sesquiterpenes were hardly spotted in these species (Sawamura et al., 2004).

Literature survey on the chemical constituents of *C. reticulata* essential oil revealed a great variability, which may have been due to several factors, among the geographical location, season and environmental factors, as well as the part of the plant used and extraction method. Nagpur suntra or santra has thin rind separated very easily. The number of segments is 10-11 with abundant juice, excellent flavor and sweet taste. To the best of the authors' knowledge, no detailed research on the volatile components of santra mandarin growing in Egypt is available. The waste of fruit rind results from edible consumption or industrial process of making juices and lemonade while the leaves from annual cutting of stems and pruning process. Therefore, the aim of this study is to investigate the chemical profile of the fruit peel and leaf oils. The anti-inflammatory and antioxidant activities of the extracted oils were also evaluated.

MATERIALS AND METHODS

The fresh leaves and fruit peel of *C. reticulata* Blanco cv. Santra (santra mandarin) were collected from the Research Station of the Faculty of Agriculture, Banha University, Egypt in January 2014. The plant was kindly identified by Dr. B. Holyel, Professor of Pomology, Faculty of Agriculture, Banha University. Voucher specimens (accession no. CR-133) were deposited in the Herbarium of the Department of Pharmacognosy, Faculty of

Pharmacy, Zagazig University.

Isolation of the essential oils

The fresh leaves and fruit peel (100 g each), were separately hydrodistilled using Clevenger-type apparatus for six hours producing oils with 1 and 2.5% yield, respectively. The oils were dried over anhydrous sodium sulphate and kept at 4°C until further analyses.

Gas liquid chromatography (GLC) and gas chromatography/mass spectrometry (GLC/MS) analysis

Procedures as described elsewhere (Hamdan et al., 2013a, b) were used for GLC and GLC/MS analysis of peel and leaf oils. The experiment was repeated three times on three independent oil samples to insure reproducibility of the results.

Identification of the essential oil components

Compounds were identified by comparing their spectral data and retention indices with literature (El-Shazly et al., 2004a, b; Adams, 2007; Hamdan et al., 2010, 2013a, b). The identified constituents are listed in the order of their elution in Table 1.

Preparation of samples for biological activity

Stock solutions of leaf and peel oils (10 mg/ml) were prepared and diluted with DMSO to reach 100 μ g/ml. Each sample was repeated in triplicate; mean and standard error of mean were calculated.

Anti-inflammatory properties

Estimation of tumor necrosis factor- α (TNF- α)

TNF- α was measured according to produces described in previous publication (Hamdan et al., 2013b, c).

Estimation of nitric oxide (NO)

Assay of nitrite accumulation, as an indicator of NO production, was estimated in macrophage (RAW 264.7) cell lysates based on the Griess reaction according to Green et al.(1982).

Determination of antioxidant properties

Estimation of superoxide dismutase (SOD) activity

The SOD activity was evaluated in macrophage (RAW 264.7) cell lysates using the nitroblue tetrazolium/phenazinemethosulfate (NBT/PMS) assay according to Ewing and Janero (1995). The cells were treated with the samples (100 μ g/ml) for 48 h and compared with control cells.

Scavenging of DPPH free radicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable deep violet radical due to its unpaired electron. The presence of an antioxidant radical scavenger can donate an electron to DPPH resulting in

decolourization of the deep violet color to pale yellow (Ratty et al., 1988). The change in colour and the subsequent fall in absorbance are monitored spectrophotometrically at 520 nm. Ascorbic acid was used as a positive control due to its known strong scavenging activity of DPPH. The half maximal scavenging capacity (SC_{50}) values for each tested sample (100 $\mu\text{g/ml}$) and ascorbic acid was estimated via dose curve and used as an indicator of activity.

Statistical analysis

All experiments were carried out three times. Statistical analysis was carried out using Student's unpaired *t*-test. Effects are considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The potential use of plant essential oils in pharmaceuticals industries has created a center of attention recently. The pharmaceutical applications of these oils may be attributed to their scientifically approved medicinal and pharmacological activities. The volatile constituent of a plant can dramatically differ according to the plant location and environment (Figueiredo et al., 2008). Generally, the machinery for production of several volatile components could be located in the plant, however it will not be used until needed according to the plant's ecosystem and surrounding conditions. Therefore, it is expected for the volatile components of a plant to differ in constituents and concentration, even between different cultivars of the same plant.

Volatile components

Volatile components from the leaf and fruit peel of santra mandarin cultivated in Egypt were separated and identified using GLC and GLC/MS and their relative abundance is listed according to their retention indices in Table 1. Altogether, 131 compounds were identified in the fruit peel and leaf oils represent 95.98 and 94.06% of total oil components, respectively. Monoterpene hydrocarbons constitute the major part of fruit peel oil (99.6%), while in the leaf oil, monoterpene and sesquiterpene hydrocarbons accounted for 82.3 and 17.7%, respectively. Limonene (79.64%) constitutes the major components of the fruit peel oil, while sabinene (23.1%) and linalool (21.2%) are the major constituents in the leaf oil. The most abundant monoterpene in leaf oil were (*E*)- β -ocimene (6.40%), terpinen-4-ol (6.32%), limonene (3.53%), γ -terpinene (2.92%), myrcene (2.86%), thymol (2.46%) and α -fenchene (1.80%). Similarly, γ -terpinene (6.52%), sabinene (2.30%), α -fenchene (1.21%), and β -pinene (1.08%) and linalool (1.02%) represent the most abundant monoterpene hydrocarbons in the fruit peel oil. Oxygenated monoterpenes have relatively low concentrations as

compared to non-oxygenated monoterpenes hydrocarbons. They possess higher percentages in leaf oil (7.10%) than in fruit peel oil (0.81%). The alcoholic monoterpenes is almost represented by linalool and Terpinen-4-ol in both leaf and fruit peel oil (Figure 1 and Table 1).

The sesquiterpene fraction is hardly presented in the fruit peel oil (0.46%) when compared with leaf oil (17.70%). β -Sinensal (3.54%), α -sinensal (1.66%), α -selinene (2.61%) germacrene B (1.17%), β -elemene (0.73%) and γ -elemene (0.83%) are the most abundant sesquiterpenes in leaf oil. Sixty seven components were unique to the leaf oil, among them, the major ones are β -sinensal (3.54%) and α -selinene (2.61%) and twenty one components were only found in fruit peel oil, however, these compounds do not represent any majors in the oil. In the light of *C. reticulata* cultivar chemo-classification by Lota et al. (2000), it is concluded that the leaf oil of santra mandarin is located under the sabinene/linalool chemotype (23.1% and 21.2%, respectively) in subclass I, while the fruit peel oil can be classified under the limonene chemotype (79.64%) sub class II.

The presence of sesquiterpenes in leaf oil by 17.70% is a major difference between the Egyptian santra mandarin and other plant cultivars (Lota et al., 2000; Chutia et al., 2009; Singh et al., 2010; Sultana et al., 2012). Sesquiterpenes are known for many ecological roles in plants. These include allelopathy with other plants, insects and microbes. Some sesquiterpene lactones are known for their antimicrobial and antifungal activities, whereas others protect the plant from environmental stresses that would cause damage. Sesquiterpenes can also act as phytoalexins, antifeedants to deter herbivores, hormones and in protection against UV (Chadwick et al., 2013).

Biosynthesis of oil components

The difference and distribution of oil components between both fruit peel and leaf oils emphasizes on the concept of organ specificity in plant genes expression. Many genes in the plant are switched on or off according to the position of the secretory tissues in the plant and this is connected to the function of the oil in the place of secretion (Sullivan et al., 2005). In this study, oils produced in leaf and fruit peel showed dramatic difference in constituents and concentration. It is evident that Geranyl-PP is the biosynthetic precursor of monoterpenes, the fruit peels' oil showed a majority of monocyclic monoterpenes such as limonene (79.64%), however the leaf oil showed more diversity in essential oils components ranging from acyclic monoterpenes (myrcene 2.86%, (*E*)- β -ocimene 6.4%, linalool 21.2%), monocyclic monoterpenes (limonene 3.5%, terpinen-4-ol 6.32%), bicyclic monoterpenes (sabinene 23.1%), aromatic monoterpenes (thymol-methyl ether 2.46%) and

Table 1. Volatile constituents from leaf and fruit peel oils of santra mandarin.

Compound	RI	Peak area percentage	
		Leaf oil	Fruit peel oil
Heptanol	902	-	0.09
Tricycline	928	0.01	0.66
α -Thujene	929	0.04	0.07
α - Pinene	938	0.60	0.35
α - Fenchene	949	1.80	1.21
Camphene	953	0.11	0.12
Verbenene	966	-	0.10
β -Pinene	973	0.13	1.08
Sabinene	978	23.1	2.30
β -Myrcene	989	2.86	tr.
<i>n</i> - Octanal	998	0.14	0.50
<i>n</i> - Decane	1000	0.02	-
δ -2- Carene	1003	0.03	0.18
<i>iso</i> - Sylvestrene	1008	0.81	-
α -Terpinene	1017	-	0.02
ρ - Cymene	1022	0.57	-
Limonene	1029	3.53	79.64
(<i>Z</i>)- β - Ocimene	1035	0.38	0.28
(<i>E</i>)- β - Ocimene	1048	6.40	0.02
γ -Terpinene	1058	2.92	6.52
<i>cis</i> - Sabinene hydrate	1063	0.60	-
ρ -Mentha-3,8-diene	1072	-	0.10
Terpinolene	1088	0.56	0.47
ρ - Cymenene	1090	0.49	-
Linalool	1096	21.2	1.02
<i>n</i> - Nonanal	1100	-	0.13
<i>cis</i> - ρ -Menth-2-en-1-ol	1121	0.36	0.05
<i>allo</i> -Ocimene	1132	0.10	0.03
<i>trans</i> - ρ -Menth-2-en-1-ol	1139	0.34	0.13
(<i>E</i>) Tagetone	1144	-	0.04
<i>trans</i> - Verbenol	1144	0.04	-
Citronellal	1153	0.05	0.25
Terpinen-4-ol	1175	6.32	0.35
ρ - Cymen-8-ol	1182	0.02	-
α - Terpeneol	1186	0.36	0.43
Myrtenol	1195	-	0.02
<i>cis</i> - Piperitol	1195	0.13	-
<i>trans</i> -Dihydrocarvone	1200	-	0.52
<i>trans</i> - Piperitol	1208	0.22	-
<i>trans</i> - Carveol	1216	-	0.07
Citronellol	1225	-	0.14
<i>cis</i> - Carveol	1229	0.03	-
Thymol, methyl ether	1233	2.46	0.07
Carvone	1243	-	0.06
Isogeijerene C	1248	0.10	-
Geraniol	1252	0.08	-
(<i>2E</i>)-Decenal	1262	-	0.05
Geranial	1268	0.05	-
Perilla aldehyde	1271	-	0.25

Table 1. Cont'd.

α - Terpinen-7-al	1285	0.06	-
Carvacrol	1298	0.16	0.11
n-Tridecane	1300	-	0.02
Undecanal	1304	0.15	0.03
p-vinyl- Guaiacol	1309	0.08	-
Methyl geranate	1324	0.30	-
δ - Elemene	1338	0.89	0.12
α -Terpinyl acetate	1349	0.04	-
Citronellyl acetate	1352	-	0.06
Neryl acetate	1360	tr.	0.05
Carvacrol acetate	1370	0.30	0.01
Geranyl acetate	1381	-	0.03
1-Tetradecene	1388	-	0.02
β - Elemene	1390	0.73	-
n-Tetradecene	1400	-	0.02
α - Funebrene	1402	0.06	-
Dodecanal	1408	-	0.10
α -cis- Bergamotene	1412	0.99	-
γ - Elemene	1436	0.83	-
Aromadendrene	1441	tr.	-
(Z)- β - Farnesene	1442	0.05	-
cis-Prenylimponene	1445	-	0.02
α - Humulene	1454	0.3	0.01
(E)- β - Farnesene	1457	0.67	-
α - Gurjunene	1477	0.59	-
γ - Muurolene	1479	tr.	0.06
α - Amorphene	1484	0.03	-
Germacrene D	1486	0.02	-
α - Selinene	1498	2.61	-
α - Muurolene	1499	0.06	-
γ - Patchoulene	1502	0.12	-
β - Bisabolene	1506	0.03	0.03
Germacrene A	1510	0.21	-
δ - Amorphene	1512	0.10	-
α - Alaskene	1513	0.08	-
δ - Cadinene	1523	0.35	0.02
Citronellyl butanoate	1531	0.04	-
cis- Sesquisabinene hydrate	1544	0.28	-
Elemol	1549	0.07	-
Germacrene B	1561	1.17	0.05
(E)- Nerolidol	1565	0.35	-
Spathulenol	1576	0.30	-
trans- Sesquisabinene hydrate	1580	0.32	-
Gleenol	1587	0.02	-
Globulol	1589	0.19	-
Rosifoliol	1599	0.08	-
n-Hexadecane	1600	-	0.01
Humulene epoxide II	1608	0.02	-
cis-Isolongifolanone	1612	0.02	-
Isolonfifolan-7- α -ol	1619	0.12	-
Muurola-4,10(14)dien-1- β -ol	1629	0.30	-

Table 1. Cont'd.

<i>allo</i> -Aromadendrene epoxide	1638	0.12	-
<i>epi</i> - α -Muurolol	1642	0.40	-
α -Muurolol	1647	0.10	-
β -Eudesmol	1650	0.12	-
α -Cadinol	1654	0.45	-
(<i>Z</i>)- α -Santalol	1675	0.28	-
β -Sinensal	1700	3.54	-
<i>n</i> -Heptadecane	1700	-	0.01
(<i>E</i>)-Apritone	1708	0.04	-
(<i>2E,6Z</i>)-Farnesal	1713	0.01	-
(<i>2Z,6E</i>)-Farnesal	1716	0.03	-
(<i>2Z,6E</i>)-Farnesol	1724	0.02	0.01
α -Sinensal	1757	1.66	0.02
(<i>Z</i>)- α -Santalol acetate	1776	0.02	-
(<i>2Z,6E</i>)-Farnesyl acetate	1823	tr.	-
(<i>2E,6E</i>)-Farnesyl acetate	1846	0.12	-
<i>n</i> -Nonadecane	1900	0.05	-
Phytol	1943	0.03	-
(<i>6Z,10E</i>)Pseudo phytol	2030	0.02	-
<i>n</i> -Heneicosane	2100	0.02	-
Methyl octadecanoate	2123	0.32	-
Oleic acid	2141	0.02	-
Incensole	2159	0.02	-
<i>n</i> -Tricosane	2300	0.04	-
<i>n</i> -Tetracosane	2400	0.05	0.03
Labd-(<i>13E</i>)-8,15-diol	2428	0.03	0.02
<i>n</i> -Pentacosane	2502	0.01	0.02
Hexacosane	2600	0.02	0.02
Heptacosane	2700	0.01	0.01
Octacosane	2800	tr.	0.01
Nonacosane	2900	0.01	tr.
Acyclic monoterpene hydrocarbons		30.86	1.23
Monocyclic monoterpene hydrocarbons		9.85	79.99
Bicyclic monoterpene hydrocarbons		28.91	11.88
Aromatic monoterpene hydrocarbons		2.46	0.07
Oxygenated monoterpene hydrocarbons		6.68	0.78
Sesquiterpenes		16.64	0.45
Others		5.34	2.36
Total		94.06	95.98

In elution order from RTX-5MS® column. RI = retention index relative to standard *n*-alkanes. tr. = trace (<0.01 %). - = not detected

sesquiterpenes (α -selinene 2.61%, germacrene B 1.17%, β -sinensal 3.54%, α -sinensal 1.66%) (Figure 1 and Table 1). Essential oil biosynthesis in leaf involve the induction of many enzymes such as myrcene synthase, pinene synthase and sesquiterpene-production enzymes as well as limonene synthase, however these enzymes were down regulated in the fruit peel tissues with the exception

of limonene synthase (Figure 1). Furthermore, the number and concentration of oxygenated components, such as linalool (21.2%) and terpinen-4-ol (6.32%) in the leaf oil more reflect the induction of the oxygenation mechanism in leaf and the absence of such mechanism in fruit peel tissues (Figure 1). Further studies should take place in future in order to confirm the expression of genes

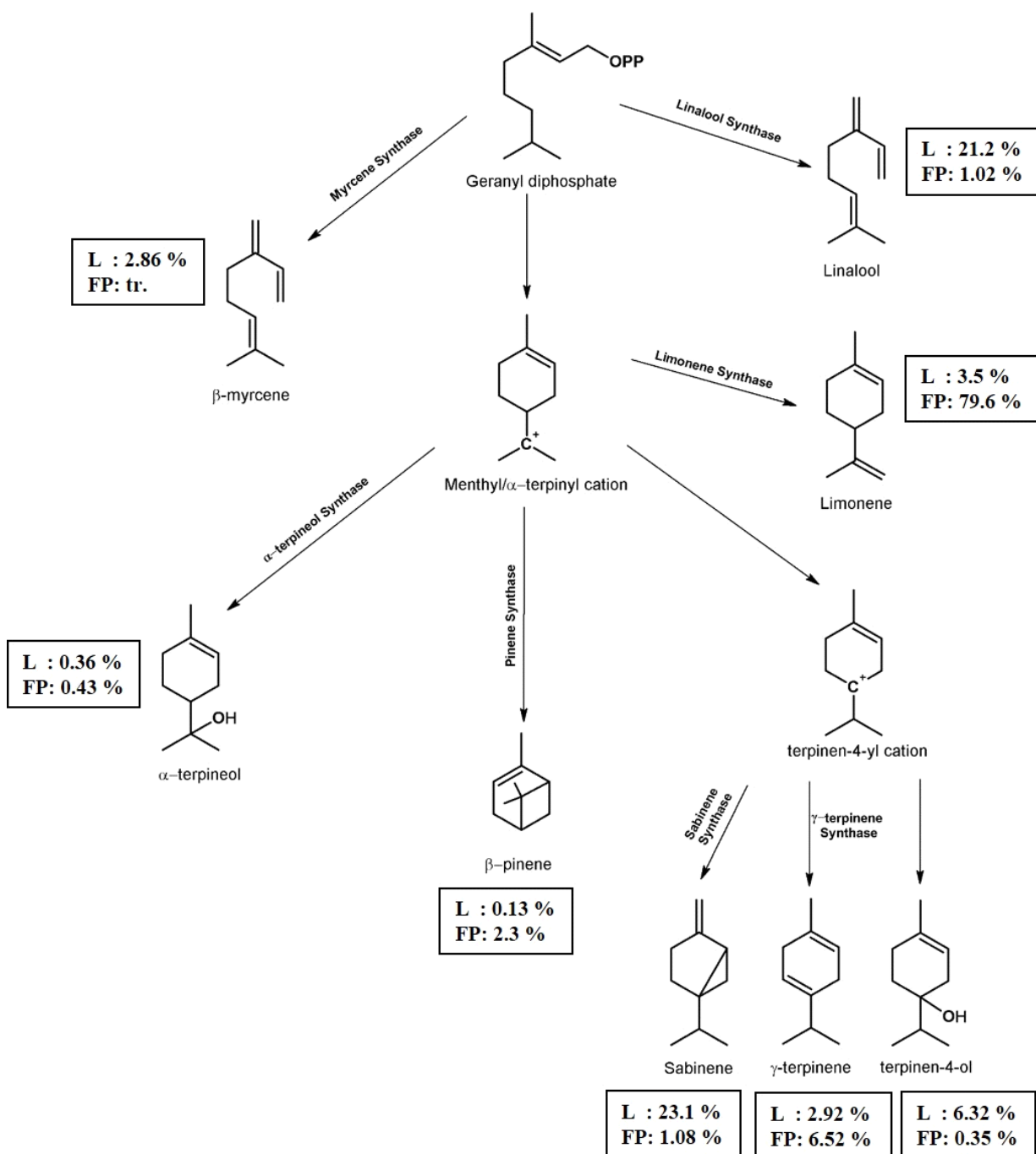


Figure 1. Biosynthesis cascade of the most common monoterpenes in santra oil. The rectangles beside the chemical structure refer to the concentration (area percentage) of this material in both leaf (L) and fruit peel (FP) oil (Dewick, 2009).

mentioned above through the uses of molecular biology tools such as real time PCR and Western plot analysis.

Anti-inflammatory activity

The anti-inflammatory activity of santra mandarin oils was

measured relative to its ability to inhibit both TNF- α and NO models. TNF- α is a pro-inflammatory cytokine which is related to inflammation and inflammatory disorders (Palladino et al., 2003). LPS induced TNF- α production reached up to 50-folds in the control. Both the leaf and fruit peel oils at concentration of 100 μ g/ml, possessed a

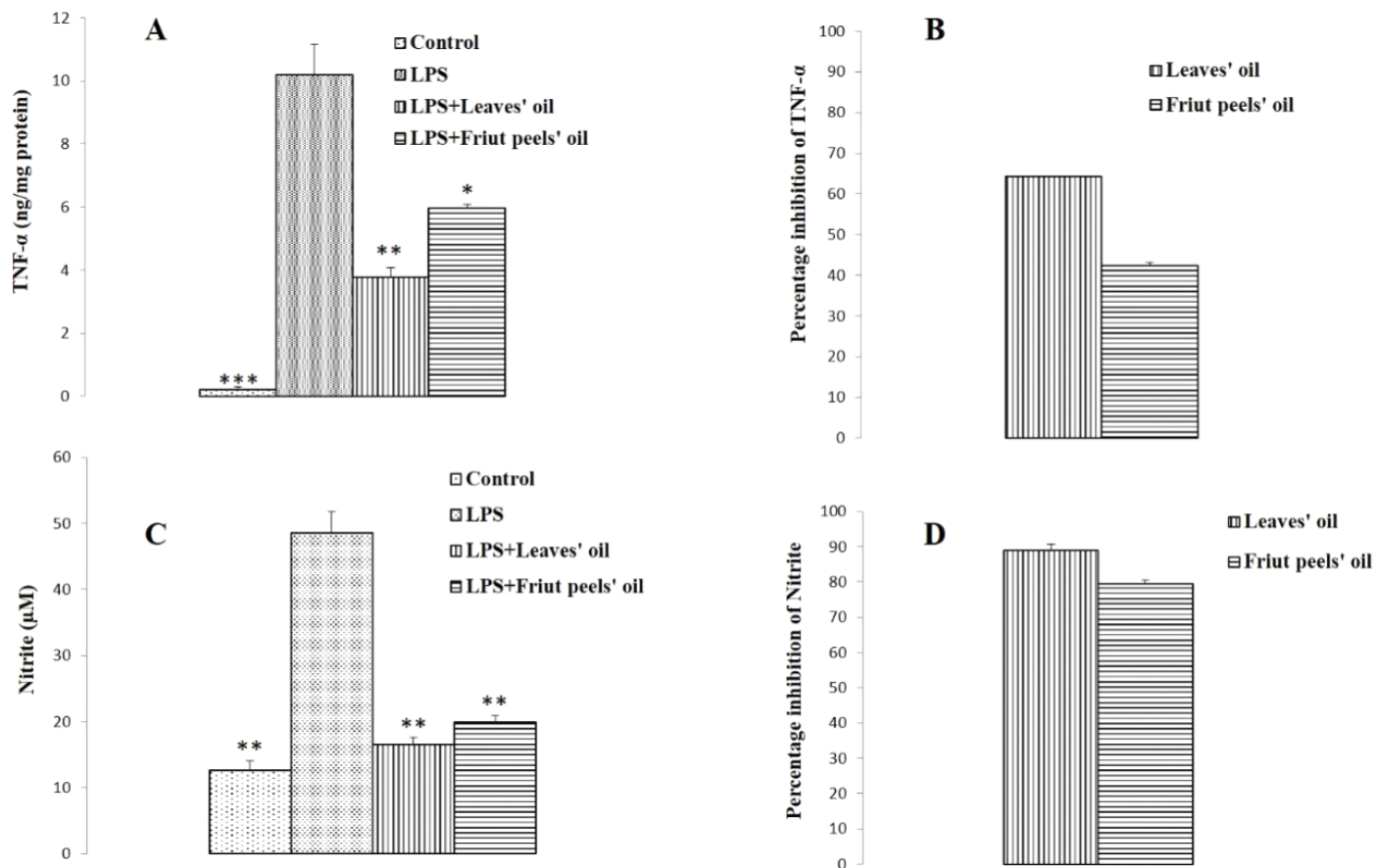


Figure 2. The anti-inflammatory activity of santra mandarin oils. A: The level of TNF- α protein in RAW 264.7 cells lysate after treatment with the samples (100 μ g/ml) for 48 h as compared to the LPS treated cells, as measured by ELISA assay. B: The percentage inhibition of TNF- α by different oil samples. C: The level of NO in RAW 264.7 cells lysate after treatment with the samples (100 μ g/ml) for 48 h as compared to LPS treated cells, as measured by Griess assay. D: The percentage inhibition of NO by different oil samples. The data are presented as absorbance (mean \pm SE) of three replicates ($n=3$). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ when compared with LPS untreated values

very high significant inhibitory activity for LPS-stimulated TNF- α level ($P < 0.001$), as shown in Figure 2. The leaf oil showed high significant reached 64.20% ($P < 0.05$) (Figure 2).

NO is an important inflammatory regulator especially in hepatic inflammatory conditions and measuring NO production is a method for assessing the anti-inflammatory effects of essential oils (Kierner et al., 2002). LPS made nearly two-fold induction of nitric oxide of the control as shown in Figure 2. 100 μ g/ml leaf or fruit peel oils possessed high significant inhibitory activity against LPS-induced NO ($P < 0.001$) to the extent similar to the control level (Figure 2). Both leaf and fruit peel oils showed significant inhibitory activity (88.87 and 79.60%, respectively) against LPS-induced NO ($P < 0.01$) (Figure 2). Santra mandarin oils reduced the levels of TNF- α and NO in Raw murine macrophage cell culture (RAW 264.7) induced by LPS (Figure 1A, B, C and D). The leaf oil

inhibitory activity for LPS-stimulated TNF- α level which showed the higher inhibition activity followed by the fruit peel oil. The anti-inflammatory activity of the fruit peel oil can be attributed to the presence of high concentration of limonene (79.64%), which is reported to suppress the production of TNF- α and NO (Yoon et al., 2010).

Although, the concentration of limonene in leaf oil (3.5%) was extremely lower than that in fruit peel oil, the leaf oils showed better anti-inflammatory effect than the fruit peel oil. This activity can be assigned to constituents which show higher concentration in the leaf oil such as sabinene (23.1%), myrcene (2.86%), (*E*)- β -ocimene (6.4%), linalool (21.2%), terpinen-4-ol (6.32%), thymol-methyl ether (2.46%), α -selinene (2.61%), germacrene B (1.17%), β -sinensal (3.54%), α -sinensal (1.66%). Germacrenes are known for their cytotoxic and anti-inflammatory activities (Adio, 2009; Vandermoten et al., 2011). Linalool is also known for its potent anti-

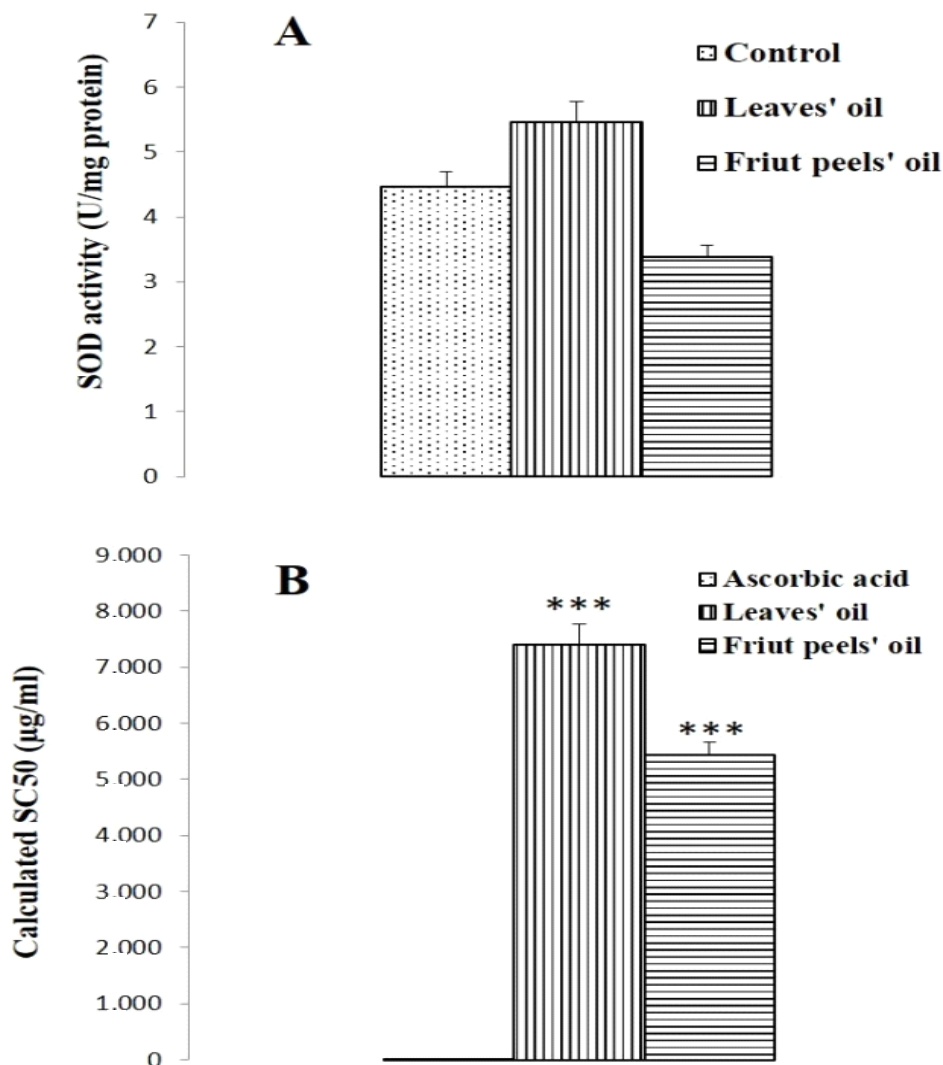


Figure 3. The antioxidant analysis of santra mandarin oils. A: The level of SOD in RAW 264.7 cells lysate after treatment with the samples (100 µg/ml) for 48 h when compared with control cells, as measured by NBT/PMS assay. The data are presented as U/mg protein (mean ±SE) of three replicates ($n=3$). B: DPPH scavenging activity of different oil samples. The data are presented as SC₅₀ (µg/ml), (mean ±SE) of three replicates ($n=3$). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ when compared with control or ascorbic acid.

inflammatory activity (Peana et al., 2002; Huo et al., effect on inhibition of edema formation (de Cassia da Silveira e Sa et al., 2013). The combination of all these oil components may have synergistic effect, making the leaf oil more potent as an anti-inflammatory agent than the fruit peel oil. To the knowledge of the authors, this is the first report on the anti-inflammatory activity of Santra mandarin volatile oils.

Antioxidant activity

The antioxidant activity of santra mandarin oils was

investigated by estimation of SOD and DPPH activities. The results indicated that there was a non-significant change in the level of SOD ($P > 0.05$) in the cells treated with different tested samples in comparison with control level as indicated in Figure 3A. The DPPH assay examines the ability of the tested oil to scavenge free nitrogenous radicals in comparison with the very well known antioxidant vitamin C. The antioxidant activity of the fruit peel and leaf oils were investigated by measuring their affinity to scavenge DPPH radicals. Ascorbic acid was used as a positive control due to its known strong scavenging activity of DPPH radicals and the results indicated that its SC₅₀ value was found to be 17.21 µg/ml

as shown in Figure 3B. The tested oils revealed no antioxidant affinity towards DPPH radicals as concluded from their high SC_{50} values ($>250 \mu\text{g/ml}$). The fruit peel oil of *C. reticulata* was reported to have an antioxidant activity (Shahzad et al., 2009; Gao et al., 2011) through the use of many *in vitro* methods such as ABST, DPPH and ferric-reducing antioxidant power (FRAP) assay. The antioxidant activity of the leaf oil has not been reported. This study indicated that the oil of both the fruit peel and the leaf of santra mandarin have weak antioxidant power in therapeutic doses ($>250 \mu\text{g/ml}$). Although, limonene is the major constituent in all *C. reticulata* fruit peel oils, the antioxidant activity of the oil differ from one cultivar to another and the reason for this is not clear and needs more investigations. These results could be attributed to other components of the oils (Lis-Balchin et al., 1998).

Conclusion

As a step towards the reuse and recycle of these by-products, the authors have herein demonstrated the chemical composition of the essential oils samples from the leaf and peel of Egyptian variety of *C. reticulata* (santra mandarin). Hydro-distilled oils from the fruit peel and leaf of santra mandarin were analyzed by GC-MS and many components of the oils were identified on the basis of their mass analysis and retention indices. Sabinene (23.1%) constitutes the major component of the leaf oil followed by linalool (21.2%). In the fruit peel oil, limonene was represented by 79.64%. The oils showed good anti-inflammatory activities calculated as their effect on TNF- α and NO; nevertheless they fail to show any antioxidant effect investigated by estimation of SOD and DPPH activities. Together with using santra mandarin oils as a good anti-inflammatory agent, they can also be used as a source of limonene, sabinene and linalool. These components are valuable with many medicinal actions and industrial uses as in cosmetic, perfumes and flavoring agent in food manufacturing.

Conflict of interests

The authors have not declared any conflict of interest.

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