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Chemical Composition and Antioxidant Potential of Essential Oils from Peels of Four Citrus Species

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ABSTRACT

Essential oils were isolated from the dried peels of *Citrus limon*, *Citrus sinensis*, *Citrus auratifolia* and *Citrus reticulata* using hydrodistillation method and were characterized using Gas Chromatography-Mass Spectrometry, while DPPH scavenging analysis was used to determine the antioxidant potentials of the oils. The hydrodistillation of the peels afforded oil in the yield of 0.61, 0.42, 0.72 and 0.52 (% w/w) for *C. limon, C. auratifolia, C. sinensis* and *C. reticulate respectively.* Analyses of the oils revealed the presence of monoterpenoids, sesquiterpenoids and non-terpenes. A total of 19, 25, 16 and 21 compounds were identified in the peels essential oils *C. limon, C. auratifolia, C. sinensis* and *C. reticulata* respectively. D-limonene, with the percentage composition of 84.9%, 44.6%, 91.8% and 82.4% in *C. limon, C. auratifolia, C. sinensis* and *C. reticulata* respectively was the most predominant compound. All the citrus species peels essential oils showed antioxidant activity of scavenging DPPH with IC_{50} of 27.29, 28.67, 32.0 and 33.0 µg/ml for *C. limon, C. auratifolia, C. sinensis* and *C. reticulata respectively*. The oils were of d-limonene chemotype and can thus be candidates for antioxidant drugs.

Key Words: Antioxidants, Essential oils, Free radical, Citrus peel oils, Limonene

1. INTRODUCTION

Imbalance between production and accumulation of reactive oxygen species (ROS) in cells and tissues in the body is as a result of phenomenon known as oxidative stress. Reactive oxygen species (ROS) otherwise known as free radicals are oxygen-containing molecules with an uneven number of electrons. The uneven number of electrons allows them to easily react with other molecules. Free radicals can cause large chain chemical reactions in the body because they react so easily with other molecules. These reactions are called oxidation. They can be beneficial or harmful (Pizzino, *et al*., 2017).

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Some synthetic drugs have been reported to be viable in the treatment of induced oxidative stress and its complications. The most common synthetic antioxidants in the food industry are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ). In addition, 2-naphthol (2NL), 4-phenylphenol (OPP) and 2,4-dichlorophenoxyacetic acid (2,4-DA) are the ones commonly used in fruits and vegetables (Usman *et al.,* 2017; García-García, and Searle, 2016; Lourenço *et al*., 2019). However, there are several published studies indicating a relationship between the long-term usage of these synthetic antioxidants and some health issues, such as skin allergies, gastrointestinal tract problems, liver damage, unfavourable reactions like nausea, anaphylaxis reaction and polydipsia. Some are carcinogenic, not readily accessible to the users and are very expensive (Ito *et al.,* 1983; Lourenço *et al*., 2019). This may result in aging, cancer, heart diseases and other disease conditions (Azad *et al*., 2008; Gope *et al*., 2014; Sahreen *et al*., 2014). Food and fruits are known to contain antioxidants that are linked to in vivo protection from oxidative stress (Oikeh *et al*., 2015).

Citrus plants belong to the family *Rutaceae*. There are about 17 species found throughout the tropical, subtropical and temperate regions (Shaw, 1979; Davies and Albrigo, 1994). Among the species are, *C. aurantium, C. microcarpa C. limon, C. auratifolia, C. sinensis, C. reticulata* (Ghasemia *et al*., 2009; Lv *et al*., 2015; Liew *et al*., 2018; Azman *et al*., 2019; Deeksha *et al*., 2020). Several of these plant species have been reported to be essential in phytochemistry and are important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Ghasemia *et al*., 2009). Thus, citrus plantations have been considered the most valuable for industrial and commercial agricultural practices in the world (Ahmed *et al.,* 2006). The citrus peel which is almost one half of the total fruit mass is a rich source of phenolic compounds (Calabro *et al.,* 2004). Many plants of the Rutaceae family have been a subject of chemical investigations for many years and empirically, several secondary metabolites have been reported in the oils of several species of the plant (Hafizu and Umar, 2017). These compounds play some excellent roles in physiological, ecological, as well as in foods and pharmaceuticals applications (Hafizu and Umar, 2017). Historically, citrus peels have been found useful in traditional medicinal applications in some countries such as China, Korea and Japan. It is believed to have cured skin inflammation, indigestion, cough, muscle pain, ringworm infections and reduce blood pressure (Lv *et al.,* 2015). For instance polyphenols, flavonoids and tannins have been reported from the extract of parts of *C. limon,* fruits of *C. aurantium,* pulp of *C. macroptera* (Gope *et al*., 2014; Divya, *et al.*, 2016; Makni *et al.,* 2018). In addition, alkaloids, phenols, flavonoids,

steroids, terpenoids, reducing sugar, saponins and cardiac glycosides were reported from the juice extracts of some citrus species (*C. tangerine* (tangerine), *C. paradisi* (grape), *C. limon* (lemon), and *C. aurantifolia* (lime)) (Oikeh *et al*., 2015)

Lawal *et al.,* (2014) had investigated the essential oil contents of C. *auratifolia* from two locations in South West Nigeria. They reported that the essential oil contents of C. *auratifolia* from Ijanikin contain caryophyllene oxide, caryophylla-3(15),7(14)-dien-6-ol, α-pinene and 2,6-dimethyl-1,5,7-octatrien-3-ol while the Ikotun sample was rich in limonene and geranial. In the same vein, Anis *et al.,* (2017), reported that limonene and β-pinene were the dominant compounds in *C. limon* and are responsible for the antimicrobial and antioxidant activity of the plant. Empirically, these four Citrus species (*C. limon, C. auratifolia, C. sinensis, C. reticulata*) contain similar compounds as observed from several studies and these compounds were responsible for their various activities which are comparable to synthetic drugs and in some cases give better activities (Mohanapriya *et al*., 2013; Lawal *et al.,* 2014; Oikeh *et al*., 2015; Onyilofe *et al*., 2015; Olatunya and Akintayo, 2017; Jubril *et al*., 2015 and Maha *et al*., 2018). Because of the unfavorable health issues associated with the long-term usage of synthetic antioxidants and the inaccessibility to the users there is the need for continuous search for safer, more effective, readily available and cheaper antioxidants from plants. It is on this basis that this research is focused on investigating the antioxidant potential of essential oils from the peels of *C. limon, C. auratifolia, C. sinensis, C. reticulata*.

2. MATERIAL AND METHODS

2.1 Collection and Preparation of Samples

Fruits of *C. limon, C. auratifolia, C. sinensis,* and *C. reticulata* were obtained from the farm of University of Ilorin in June 2018. The fruits were identified at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria. The peels of the citrus fruits were removed, dried and pulverized.

2.2 Extraction

Pulverized dried peels (500 g each) of *C. limom C. auratifolia, C. sinensis* and *C. reticulata* were separately hydrodistilled for 3 hours in a Clevenger-type apparatus following the British Pharmacopoeia Method (British Pharmacopoeia 2012). The resulting oils were collected and preserved in a sealed sample bottle and stored under refrigeration until analysis was done

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC analyses of the oils were performed separately on an Orion micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with Cp-Sil 5 and Cp-Sil 19 (fused silica, 25 x 0.25 mm, 0.15 µm film thickness) and flame ionization detector (FID). The volume of each of the oils injected was 0.2 mL, and the split ratio was 1:25. Oven temperature was programmed from 60-260^{o}C at 2 ^{o}C/min using hydrogen as carrier gas. Injection and detector temperatures were maintained at 250^{o}C and 260^{o}C, respectively. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factors.

The identification of the components was based on comparison of retention indices (determined relative to the retention times of series of n- alkanes) and mass spectra with those of authentic samples and with data from literature (Jenning and Shibamoto, 1980; Joulain and König, 2018; Adams 2012).

2.4 DPPH Assay

Antioxidant activity of the oils in terms of radical scavenging or hydrogen donating ability was measured, using a modified method of Clarke, (2013). In this method, 1.5 ml of 2, 2-diphenyl-1-picryl-hydrazil, DPPH, solution (10^{-4} M, in 95% Ethanol) was incubated with 1.5 ml of the oil separately at various concentrations (6.25-100 µg/ml). The reaction mixture was shaken thoroughly and incubated in the dark for 30 mins at room temperature. The control was prepared using same method as stated above but with the exception of the extract. Absorbance of the solution was measured at 517 nm using UV-spectrophotometer. The radical scavenging activity (RSA) was measured by determining the absorbance of the mixture. The assay was carried out in triplicate and the result were expressed in mean values ± standard deviation. The concentration of the oil that gave 50% inhibition (IC_{50}) of the DPPH radical was calculated from the graph of percentage inhibition against the concentration of the oil. Ascorbic acid was used as standard. The percentage inhibition was calculated using to equation 1.

$$\% \text{ inhibition} = \frac{A_c - A_t}{A_c} \times 100 \quad (1)$$

Where A_c = the absorbance of control sample (containing all reagents except the test compound)

A_t = the absorbance of the test samples.

3. RESULTS AND DISCUSION

The essential oil yields from peels of *C. limon, C. auratifolia, C. sinensis*, and *C. reticulata* are presented in Table 1.

Table 1: Yields (% w/w) of essential oils from peels of *C. limon, C. auratifolia, C. sinensis* and *C. reticulata.*

Sample	Yield (% w/w)
C. limon	0.61
C. auratifolia	0.42
C. sinensis	0.72
C. reticulate	0.52

The yield of the oil from the peel of the four *Citrus* species ranges from 0.42 to 0.72% (w/w) with *C. auratifolia* and *C. sinensis* having the lowest and highest oil yields of 0.42 and 0.72% (w/w) respectively (Table 1). These results were lower but compared favourably with those obtained for the peel oils of seven citrus species from the Solan district of the Himachal Pradesh state in northern India investigated by Sharma and Vashist (2015). This yields are however slightly higher than those obtained by Kamal, *et al*. (2011) from peels essential oil of three citrus species (*C. reticulata, C. sinensis and C. paradisii*) obtained from Citrus orchards of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. It has been reported that variation of essential oil yield be due to genetic factors, developmental progress, plant origin, harvesting method, drying and storage methods, extraction and analysis methods (Daghbouche et al. 2020), thus the slight variations in the yield could be due to phenotypic differences of the citrus species.

Table 2 presents the retention indices, relative percentages and identities of constituents of the oil from the four citrus species. A total of 16, 19, 21 and 25 compounds representing 99.2, 97.9, 99.1 and 96 % of the oils were identified from the peels *C. sinensis, C. limon, C. reticulata* and *C. auratifolia* respectively.

Table 2: Chemical composition (%) of essential oils from peels of *C. limon*, *C. auratifolia*, *C. sinensis* and *C. reticulata*.

Compounds	KI	Percentage Composition				MS data
		C. limon 4.0	*C. auratifolia*	*C. sinensis*	*C. reticulata*	
Sabinene	897		1.4	-	0.1	121, 105, **93**, 77, 69
4-Carene	919	0.1	-	0.1	-	121, 105, **93**, 79, 67
β-Pinene	943	0.7	19.3	-	0.6	121, 105, **93**, 79, 69
3-Carene	948			0.1		121, 105, **93**, 77, 67
β-Myrcene	958	2.5	1.1	2.3	2.2	121, 107, 93, 79, 69
γ-Terpinene	968	-	-	-	9.4	121, 105, **93**, 77,66
α-Phellandrene	969	0.1	-	0.1	0.1	119, 105, **93**, 77, 65
cis-β-Ocimene	976	0.3	0.2	0.1	0.1	121, 105, **93**, 79, 67
β-Terpinene	993	-	-	0.2	-	121, 105, **93,** 77, 69
α-Terpinene	998	1.3	5.9	-	0.2	**121**, 105, 93, 77, 65
n-Caprylaldehyde	1005	-	-	0.1	0.3	100, 84, 81, **57**
d-Limonene	1018	**84.9**	**44.6**	**91.8**	**82.4**	107, 93, 79, **68**, 53
Isoterpinolene	1023		0.1	-	--	136, 1**21**, 107, 93, 79
o-Cymene	1042	0.4	9.2	-	0.5	134, **119,** 103, 91, 77
α-Terpinolen	1052	-	0.7	-	0.4	121, 105, **93**, 79, 67
Eucalyptol	1059	0.2	-	0.6	0.2	108, 93, 81, 69, **43**
2,3,5-Trimethyl-4-methylene-2-cyclopenten-1-one	1060	-	-	0.1	-	136, 121, **93**, 77, 67
β-Linalool	1082	0.5	0.7	2.1	0.8	107, 93, **71**, 69, 55
n-Nonaldehyde	1104	0.1	-	0.1	0.1	114, 98, 95, 70, **57**
Terpinen-4-ol	1137	1.3	2.5	0.1	0.1	111, 93, **71**, 69, 43
endo-Borneol	1138	-	0.1	-	-	111, 84, **81**, 69, 43
α-Terpineolene	1143	0.8	3.4	0.9	0.1	107, 93, 81, **59**, 43
p-Tolylmethylcarbinol	1169	-	-	0.1	-	136, **121**, 93, 77, 65
Lemarome	1174	0.1	1.4	-	-	123, 109, 84, 83, **69**
β-Citronellol	1179	-	0.4	-	0.1	123, 109, 95, 81, **69**
Decanal	1204		0.3	0.4	0.2	110, 95, 70, 57, **43**
cis-Geraniol	1228	0.3	0.3	-	-	121, 111, 93, 80, **69**
Carvacrol	1262	0.1	-	-	-	150, **135**, 117, 107, 91
Undecanal	1303		0.1	-	-	124, 110, 96, 82, **55**
Neryl acetate	1352	0.1	0.2	-	-	121, 107, 93, 80, **69**
(-)-cis-β-Elemene	1398	-	0.2	-	-	121, 107, 93, **81**, 68
α.-Bergamotene	1430	-	0.9	-		119, 107, **93**, 77, 69.
α.-Farnesene	1458	-	0.9	-	0.6	119, 109, **93**, 79, 69
β-**Caryophyllene**	1494	0.1	0.6	-	0.2	120, 105, **93**, 79, 69
β.-Bisabolene	1500	-	1.4	-	-	119, 109, 93, 78, **69**
Germacrene D	1515	-	0.1	-	-	204, **161**, 147, 133, 119
α-sinensal	1646				0.4	119, 107, **93**, 79, 66
Total		**97.9**	**96.0**	**99.2**	**99.1**	
Number of compounds		**19**	**25**	**16**	**21**	

In all, the compounds identified are categorized as hydrocarbon monoterpenoids, oxygenated monoterpenoids, sesquiterpenoids and non-terpenes (Table 2). For *C. limon,* a total of 19 compounds were identified in the oil, the most abundant of these is d-limonene (84.9%) which is a monoterpenoid. and other monoterpenoids in the *C. limon* oil are sabinene (4.0%), 4-carene (0.1%) β-pinene (0.7%), β-myrcene (2.5%), α-phellandrene (0.1%), α-terpinene (1.3%), cis-β-ocimene (0.3%), and o-cymene (0.4%). The oxygenated monoterpenoids include: eucalyptol (0.2%), terpinen-4-ol (1.3%) which is the predominant, α-terpineol (0.8%), lemarome (0.1%), cis-geraniol (0.3%) and carvacrol (0.1%). β-Caryophyllene (0.1%) a sesquiterpenoid was also detected while n-nonaldehyde (0.1%) and neryl acetate (0.1%) appear to be the non-terpenes present in the oil.

d-limonene (44.6%) was also found to be the predominant in the oil of *C. auratifolia,* among the 25 compounds identified. other hydrocarbons monoterpenoids in the oil include sabinene (1.4%), β-pinene (19.3%), β-myrcene (1.1%), α-terpinene (5.9%), cis-β-ocimene (0.2%), isoterpinolene (0.1%), o-cymene (0.4%) and α-terpinolene (0.7%). The oxygenated monoterpenoids are terpinen-4-ol (2.5%), endo borneol (0.1%), α-terpineol (3.4%) the predominant, lemarome (1.4%), β-citronellol (0.4%), decanal (0.3%) and cis-geraniol (0.3%). It also contains sesquiterpenoids such as α-bergamotene (0.9%), α-fernesene (0.9%), β-caryophyllene (0.6%), β-bisabolene (1.4%) which is predominant and germacrene D (0.1%). On the other hand, udecanal (0.1%) and neryl acetate (0.2%) are the non-terpenes present.

On the other hand, *C. sinensis* contained 16 compounds, of which also d-limonene (91.8%) is the predominant, other hydrocarbons monoterpenoids in the oil are 4-carene (0.1%), 3-carene (0.1%), β-myrcene (2.3%), α-phellandrene (0.1%), β-terpinene (0.2%) and cis-beta-ocimene (0.1%). The oxygenated monoterpenoids include: eucalyptol (0.6%), terpinen-4-ol (0.1%), α-terpineol (0.9%) which is the predominant and decanal (0.4%). N-caprylaldehyde (0.1%), 2,3,5-Trimethyl-4-methylene-2-cyclopenten-1-one (0.1%), n-nonaldehyde (0.1%) and p-tolylmethylcarnol (0.1%) are the non-terpenes in the oil whereas no sesquiterpenoid was found in this oil. Twenty one compounds were identified from *C. reticulata* with d-limonene (82.4%) being the most abundant. Other hydrocarbons monoterpenoids in the oil of *C. reticulata* are sabinene (0.1%), β-pinene (0.6%), β-myrcene (2.2%), γ-terpinene (9.4%), α-phellandrene (0.1%), α-terpinene (0.2%), cis-β-ocimene (0.1%), o-cymene (0.5%) and α-terpinolene (0.4%). The oxygenated monoterpenoids in *C. reticulata* include eucalyptol (0.2%), terpinen-4-ol (0.1%), α-terpineol (0.1%), β-citronellol (0.1%) and decanal (0.2%). The sesquiterpenoids are

α-fernesene (0.6%), β-caryophyllene (0.2%) and α-sinensal (0.4%), while n-caprylaldehyde (0.3%) and n-nonaldehyde are the non-terpenes in the oil.

From the results, d-limonene is the most abundant compound in the peel essential oils from *C. limon, C. auratifolia, C. sinensis* and *C. reticulata.* In the report of Hafizu and Umar, (2017), d-limonene dominated the chemical constituents of *C. sinensis.* Similarly, limonene was reported as the most prevalent chemical constituent in the essential oils of *C. reticulata, C. sinensis* and *C. paradisii* (Kamal, *et al*., 2011), as well as *C. reticulata, C. japonica* and *C. sinensis* reported by Lin *et al*., (2021). In other works monoterpenoids and sesquiterpenoids have been reported to be the dominant compounds in essential oils from citrus species and these enhance their antioxidant potential (Cakir *et al.,* 2004).

Table 3 presents the percentage DPPH inhibition of the essential oils from the four citrus species.

Table 3: Percentage Inhibition of Essential Oils from peels of *C. limon, C. sinensis, C. aurantifolia, C. reticulata* and Ascorbic Acid

Concentration (µg/ml)	**Percentage Inhibition (%)**				
	C. limon	***C. sinensis***	***C. aurantiifolia***	***C. reticulata***	**Ascorbic acid**
6.25	44.78	40.8	44.28	42.29	41.79
12.50	57.73	51.24	49.75	50.25	46.77
25.00	61.22	62.19	52.24	52.74	48.26
50.00	72.14	77.61	70.15	66.17	53.23
100.00	94.53	81.59	74.63	72.64	66.17

The Table revealed that the antioxidant activities of the peel essential oils from the citrus species are concentration dependent. The activities of the essential oils of the four citrus species increase steadily with increase in concentration. The highest and lowest activities of the oils were obtained at 100 and 6.25 µg/ml. The lowest percentage inhibitions of 40.8, 42.28, 42.29 and 44.78% were recorded for *C. sinensis, C. aurantiifolia, C .reticulata and C. limon* respectively at 6.25 µg/ml (Table 3). On the hand, the highest percentage inhibition of 94.43, 81.59, 74.63 and 72.64 were recorded for *C. limon, C. sinensis, C. aurantiifolia* and *C. reticulata* respectively at 100 µg/ml [Fig. 1]. In comparison with the standard, ascorbic acid, it is noted that the essential oils of the four citrus species recorded higher activities than the standard (Table 3).

Table 4 present the half maximal inhibitory concentration **(**IC_{50}) values of the oils. Let the table be inserted here.

Table 4: Half-maximal inhibitory concentration (IC_{50}) of essential oils from peels of *C. limon, C. sinensis, C. aurantifolia and C. reticulata* and ascorbic acid.

Sample	IC_{50}
C. limon	27.29 ±0.039
C. sinensis	28.67 ±0.035
C. auratifolia	32.00 ±0.027
C. reticulate	33.00 ±0.025
Ascorbic acid	33.58 ±0.019

The lowest and highest values of IC_{50} of 27.29 ± 0.039 and 33.00 ± 0.025 µg/ml were recorded for *C. limon,* and *C. reticulata* respectively. Though the IC_{50} of the oils are lower than the reference standard, ascorbic acid (33.58 ± 0.019 µg/ml), these values are compare favorable to that of the of the standard, Table 4. This result indicates that the oils have potentials of being developed as antioxidant agents.

The radical scavenging potential of the oils suggest that the presence of *d-limonene, β-caryophyllene* as well as the synergic action of other constituents in the oils contributed to their radical scavenging activity (Li *et al*., 2014; Bayala *et al*., 2014). However, our results showed that the oils exhibited radical scavenging capacity comparable to standard and can be used as antioxidants.

4. CONCLUSION

GC-MS analysis of essential oils from *C. sinensis, C. auratifolia, C. reticulata and C. limon* revealed the presence of medicinally valued bioactive components like d-limonene, terpinen-4-ol, α-terpineol, decanal, α-fernesene, β-caryopyllene, β-bisabolenen, carpryaldehyde, β-Myrcene, β-ocimene, terpinen-4-ol, β-linalool and neryl acetate. The results of the scavenging activity analysis of the oils also indicate that the oils have potentials of being used as antioxidant agents as their activities compared favorably with the standard, ascorbic acid. Some of the compounds identified in the oils have been linked to antioxidant activities in some plants and may be responsible for the activities of the oils.

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