Advances in Research

15(2): 1-9, 2018; Article no.AIR.41981
ISSN: 2348-0394, NLM ID: 101666096

Evaluation of Chemical Composition and Antioxidant Potential of Essential Oil from *Citrus reticulata* Fruit Peels

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Authors' contributions

This work was carried out in collaboration between both authors. Author LG managed the literature searches and analyses of the study. Author SK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2018/41981
Editor(s):
(1) Francisco Torrens, Institut Universitari de Ciència Molecular, Universitat de València, Edifici d'Instituts de Paterna, València, Spain.
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Reviewers:
(1) Daniela Hanganu, Iuliu Haţieganu University of Medicine and Pharmacy, Romania.
(2) Phyu Phyu Myint, University of Yangon, Myanmar.
Complete Peer review History: http://www.sciencedomain.org/review-history/24899

Original Research Article

Received 9th March 2018
Accepted 25th May 2018
Published 31st May 2018

ABSTRACT

Aims: To determine the chemical composition and antioxidising potential of *Citrus reticulata* fruit peel essential oil.
Study Design: Isolation of essential oil from *Citrus reticulata* fruit peel and determination of its chemical composition through GC MS studies. Nutrient and phytochemical composition of the essential oil was also determined. The essential oil was also analysed for its antioxidant potential.
Place and Duration of Study: Department of Chemistry, Punjab Agricultural University, Ludhiana (Punjab) India. Between October 2017 to March 2018.
Methodology: Isolation of essential oil from *Citrus* peels powder was carried out by hydrodistillation using a Clevenger-type apparatus. The chemical composition of essential oil was determined by Gas Chromatography-Mass Spectrometry (GC MS) studies. Proximate analysis of peel powder was carried out through methods of Association of Analytical Chemists (A.O.A.C). The essential oil was also analysed for its phytochemical composition. Antioxidant potential of *citrus* fruit peel oil was

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evaluated by a free radical scavenging effect on 1, 1 – diphenyl-2-picrylhydrazyl (DPPH).
Results: The yield of essential oil obtained from *citrus* fruit peels were found to be 0.3 ml/100 g peel powder. GC-MS studies revealed the presence of limonene (50.42%) as its major constituent. Phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, terpenoids, saponins and steroids. Proximate nutritional analysis showed the presence of (%) ash (4.2), crude fat (3.4), crude protein (4.8), crude fibre (8.3) and sugar (7.2) content. The oil showed 15.79 and 69.87% radical scavenging activity at 200 and 2000 ppm respectively which is less than the standard ascorbic acid used.
Conclusion: *Citrus* peel is a promising source of various bioactive compounds that play an important role in health promotion and disease prevention.

Keywords: Antioxidant potential; Citrus peel; essential oil; free radical; phytochemicals.

1. INTRODUCTION

Free radicals such as hydroxyl radical (OH^-), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and active oxygen species are constantly formed through normal cellular metabolism in the human body and are involved in the pathogenesis of various human ailments for instance cancer, ageing, atherosclerosis and diabetes [1]. Mammalian cells possess internal defence mechanism which consists of antioxidant compounds and various enzymes such as superoxide glutathione peroxidase, dismutase, and catalase which protect cells against higher levels of free radicals. Extra protection can be provided by the exogenous addition of certain compounds like proteins (albumin and transferin), minerals (zinc and selenium), vitamin A, E and β-carotene *etc.* [2]. Current research has confirmed that food rich in antioxidants impart a major role in the prevention of cancer [3], neurodegenerative diseases [4] and cardiovascular diseases. So the search for new compounds which contains antioxidant properties is very active field of research. In human diet, most of the antioxidant compounds are obtained from different plant sources and belong to different classes of compounds such as coumarins, tannins, phenolics, flavonoids, procyanidins and xanthones which vary in chemical and physical properties [5]. So they can be viewed as promising antioxidising agents.

Citrus belongs to the family Rutaceae and they are well-known crops with potential socio-economic influence all over the world. Their medicinal features, flavour and nutritional value are well known. *Citrus* family contains a variety of phytochemicals in various plant parts like fruits, peels, leaves, bark etc. which exhibit different biological properties such as insecticidal, seed germination and seedling growth promoters [6], antimicrobial [7] and anti-tumour [8] agents. Intake of *citrus* fruits in the human diet provides different constituents which are important for human nutrition including dietary fibres, folic acid, flavonoids, coumarins, vitamin C, potassium and pectins [9]. Various flavonoids present in *citrus* fruits and seeds have strong antioxidative and free radical scavenging activities [10,11]. *Citrus* essential oils have been used in many products such as beverages, foods, cosmetics, medical formulations and as flavouring agents. Their volatile components consist of various monoterpenes, sesquiterpene hydrocarbons and their derivatives such as acids, esters, aldehydes, ketones and alcohols [12]. The present work reports the proximate composition of *Citrus reticulata* fruit peel samples, phytochemical analysis and antioxidant potential of essential oil extracted by hydrodistillation from *Citrus reticulata* fruit peels. Antioxidant potential of essential oil was determined by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging method.

2. MATERIALS AND METHODS

2.1 Samples and Chemicals

Citrus fruits were purchased from local market of Ludhiana.Voucher specimen of fruit samples were deposited in the department of Fruit Science, PAU, Ludhiana. Different solvents and other chemicals used in the present study were of analytical grade and purchased from S.D. Fine Chem Pvt. Ltd, Mumbai. This study was conducted in department of Chemistry, PAU Ludhiana in October to March 2018. GC MS analysis was carried out from Advanced Instrumentation Research Facility, Jawarharlal Nehru University, New Delhi.

2.2 Isolation of Essential Oil

The fruit peels of *Citrus reticulata* were separated manually, dried under shade, powdered using a

blender and the isolation of oil from the powdered material (100g) was carried out by hydrodistillation for 4 hours using a Clevenger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulfate, filtered and stored in an air-tight container covered with aluminium foil at +4ºC for further use.

2.3 Gas Chromatography / Mass Spectrometry (GC/MS) Analysis of Essential Oil

GC-MS analysis of peel oil was carried out with the help of SHIMADZU GC MS QP 2010 using CARBOWAX capillary column using Helium as a carrier gas. The identification of compounds were based on a comparison of their mass spectra with those of Wiley, NBS libraries and FFNSC.

2.4 Quality Analysis of Peel Powder and Phytochemical Screening of *Citrus* Peel Essential Oil

Proximate analysis (ash, moisture, crude protein, sugar, crude fibre and crude fat content) of peel powder was carried in accordance with AOAC (2005) method [13]. Total sugar content was determined as described by Dubois et al. [14]. Essential oil of *Citrus* fruit peels was subjected to preliminary phytochemical screening to check the presence of various phytochemicals [15]. Stock solution of the oil with a concentration of 1mg/ml was prepared and used for the screening.

2.5 Testing of Antioxidant Potential

Antioxidant potential of *Citrus* fruit peel oil was evaluated by free radical scavenging effect on 1, 1 – diphenyl-2-picrylhydrazyl (DPPH) [16]. A series of concentrations of essential oil ranging from 200µg/ml to 2000 µg/ml were prepared in methanol. A standard DPPH solution containing 400 µ mole was prepared in methanol. 1 ml of each concentration of essential oil was then mixed with 3ml of a standard solution of DPPH. Incubation of mixtures was carried out in the dark for 30 minute at room temperature. The absorbance (abs) of mixtures was measured by using Perkin-Elmer 45 UV-Visible spectrometer at 517 nm. DPPH mixture without methanol was used as blank and ascorbic acid was used as a standard. Triplicate of samples were assayed. Inhibition percentage (I %) of DPPH radical by oil was calculated by using the following formula:

$$I = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of blank}}$$

2.6 Statistical Analysis

The results of proximate analysis and antioxidant activity were expressed as means ± standard error. T test using SPSS version 20 was used to evaluate data of antioxidant potential of peel essential oil with P = 0.01 accepted as significant.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

The yield of essential oil obtained from *Citrus* fruit peels was found to be 0.3 ml/100 g peel powder. It has been reported that yield of *Citrus* peel essential oil varied with individual plant species ranging from 0.2-2.0% [17]. Essential oil content from fresh, ambient and oven-dried peels of *C. sinensis, C. reticulata and C. paradissi* ranged from 0.24-1.07, 0.30-0.50 and 0.20-0.40g/100g respectively [18].

3.2 Chemical Composition of Essential Oil

GC MS studies of essential oil revealed the presence of 80 compounds in essential oil extracted from peels of *Citrus reticulate* as shown in Fig. 1. The various compounds detected along with their retention time and percentages are shown in Table 1. The various compounds detected were limonene (50.42%), n-hexadecanoic acid (5.65%), alpha sinensal (3.14%), carveol (3.09%), myrecene (3.03%), delta-cadinene (2.53%), alpha-farnesene (1.67%), beta-sinensal (1.65%), alpha-copaene (1.49%), beta-copaene (1.30%), alpha-humulene (1.23%) alpha-terpineol (1.19%), [1,1'-bicyclopentyl]-2-one (1.16%) and decanal (1.28%). Many reports on *Citrus* peel essential oil chemical composition confirmed the presence of limonene as major compound [19, 20]. Limonene is listed as a flavouring agent in the code of Fedral Regulation and is considered as safe [21]. It is used to cure gastric disorders and has an antiproliferative effect on cancer cells [22]. Antimicrobial, expectorant antiviral, sedative, and antilithic activities of limonene have also been reported [23]. The results of the chemical composition of *Citrus* peel essential oil partly

agree with the previously reported data in literature from the same species because the chemical composition of essential oils varies depending upon the age of the plant, harvesting time, geographical and ecological conditions [24,25].

Table 1. Chemical composition of *citrus* peel essential oil

Peak	Retention time	Percentace	Name of compound
1	7.708	0.27	alpha- Pinene
2	9.219	0.08	Sabinene
3	9.952	3.03	Myrcene
4	10.614	0.20	Z,Z,Z-1,4,6,9-Nonadecatetraene
5	12.238	50.42	Limonene
6	12.520	0.12	Z- Beta- Ocimene
7	12.960	0.05	gamma-Terpinene
8	13.627	0.11	n-Octanol
9	14.860	0.45	Linalool
10	15.036	0.17	n-Nonanal
11	15.831	0.77	trans-para-Mentha-2,8-dien-1-ol
12	16.501	0.73	P-Mentha-E-2,8(9)-dien-1-ol
13	16.967	0.03	3-(methoxy)-3-methyl-6-prop-1-en-2 –Cylcyclohexene
14	17.078	0.04	cis β-Terpineol
15	18.121	0.08	alpha 2,4-Cyclohexadiene-1-methanol
16	18.258	0.08	n- Octanol
17	18.465	0.45	(-)-terpinen-4-ol
18	18.884	0.13	trans-Isocarveol
19	19.226	1.19	alpha-Terpineol
20	19.465	1.16	[1,1'-Bicyclopentyl]-2-one
21	19.725	1.28	Decanal
22	19.931	0.09	Octyl-acetate
23	20.471	3.09	trans-Carveol
24	20.819	0.15	Carvomenthol
25	20.991	0.81	5-Isoprorpenyl-2-methyl-2-Cyclohexen-1-ol
26	21.384	0.50	2-methyl-5-(1-methylethene) 2-Cyclohexen-1-one
27	22.483	0.07	3-methyl-6-(1- methylethene) 2-Cyclohexen-1-one
28	22.751	0.22	Perillaldehyde
29	22.817	0.18	Undecanol
30	23.588	0.15	Limonen-10-ol
31	23.946	0.04	Perilla alcohol
32	24.271	0.12	n-Undecanal
33	24.430	0.92	4-vinyl- Guaiacol
34	25.866	0.24	alpha-Cubebene
35	26.096	0.19	3,7-dimethyl 6-Octen-1-ol
36	27.116	1.49	alpha-Copaene
37	27.325	0.06	Neryl acetate
38	27.624	0.54	beta-Copaene
39	28.281	0.06	alpha-, trans-Bergamotene
40	28.666	0.84	Dodecanal
41	28.898	0.34	(E)-Caryophyllene
42	29.336	0.06	beta-Copaene
43	29.593	0.07	alpha.-Guaiene
44	30.217	0.07	6,10-Dimethylundeca-5,9-dien-2-one
45	30.401	1.23	alpha-Humulene
46	31.100	0.07	Cadina-1,4-diene
47	31.240	0.07	gamma-Muurolene
48	31.473	1.30	beta.-Copaene

Peak	Retention time	Percentace	Name of compound
49	32.049	0.22	Bicyclogermacrene
50	32.212	0.24	alpha-Muurolene
51	32.600	1.67	(E,E)-, alpha-Farnesene
52	32.758	0.03	gamma-Cadinene
53	33.098	2.53	delta-Cadinene
54	33.233	0.20	Sesquisabinene
55	33.544	0.07	trans-Cadina-1,4-diene
56	34.246	0.79	alpha-Elemol
57	35.303	0.54	(-)-Spathulenol
58	35.846	1.28	n-Dodecanoic acid
59	36.803	0.08	Ethyl iso-allocholate
60	37.237	0.22	Epicubenol
61	37.421	0.31	gamma-Eudesmol
62	37.873	0.08	alpha.-Cadinol
63	38.311	0.99	Cadin-4-en-10-ol
64	38.722	0.15	3,7-dimethyl 6-octenal
65	39.281	0.22	Humulene
66	39.780	1.65	beta-Sinensal
67	40.973	0.05	2,6,10-trimethyl 2,6,9,11-Dodecatetraenal
68	41.842	3.14	alpha-Sinensal
69	42.120	0.19	2-pentyl-2-Nonenal
70	42.747	0.94	tetradecanoic acid
71	43.507	0.28	Nootkatone
72	44.096	0.05	Cryptomeridiol
73	47.189	0.05	Farnesyl acetone
74	47.751	0.26	methyl- Hexadecanoate
75	48.745	0.12	2-dodecen-1-yl(-) succinic anhydride
76	49.688	5.65	n-Hexadecanoic acid
77	52.933	0.32	Methyl ester 9,12-Octadecadienoic acid
78	54.591	0.94	Linoleic acid
79	54.729	0.19	Methyl Linoleate
80	54.884	0.31	Dodecenyl Succinic Anhydride
Total		95.98	

3.3 Quality and Phytochemical Analysis

Citrus peel powder was revealed for moisture, ash, crude fat, crude protein, crude fibre and sugar content determination for quality analysis (Table 2). The moisture content of the sample was found maximum (49.0±0.47%). This was followed by crude fibre content (8.3±0.42%). The sugar content was found to be 7.2±0.07% which was followed by crude protein content (4.8±0.13%). Ash content in the sample was 4.2±0.17%.and fat content was found to be least (3.4±0.14%). These results were in agreement with work reported on essential oils of *Citrus* species [26]. Hence, *Citrus* fruit peels can be used as a noble source of crude fibres having various health benefits such as their ability to reduce hypertension and hyperlipidemia, serum LDL-Cholestrol level and prevents colon cancer [27]. Phytochemical analysis of.essential oil from *Citrus reticulata* fruit peels showed the presence of terpenoids, flavonoids, alkaloids, glycosides, saponins and steroids [28]. Tannins and phenols were absent in essential oil as shown in Table 3. These phytochemicals play an important role in human health and are actively used in nutraceuticals [29,30].

Table 2. Proximate composition of *citrus* peel sample

Composition	Percentage dry weight basis
Moisture content	49.0±0.47
Ash content	4.2±0.17
Fat content	3.4±0.14
Crude protein content	4.8±0.13
Crude fibre content	8.3±0.42
Sugar content	7.2±0.07

The results were significant at P = 0.01

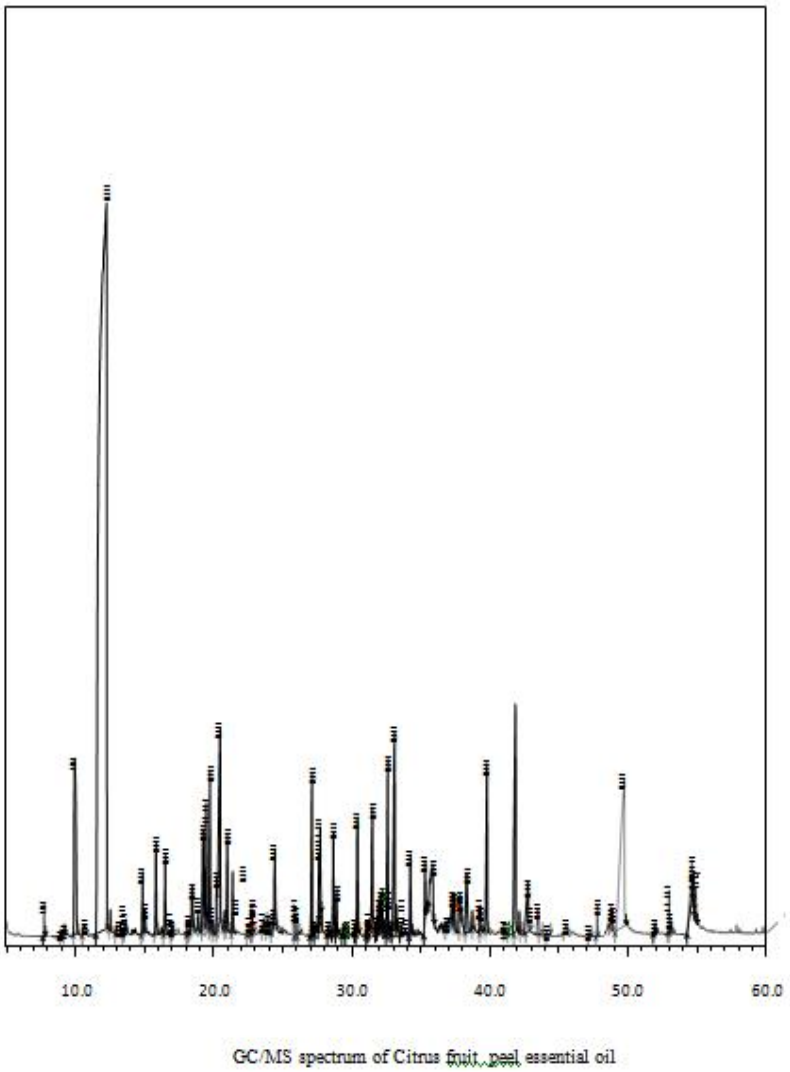

Fig. 1. GC/MS spectrum of *citrus* fruit peel essential oil

Table 3. Phytochemical constituents of *citrus* peel essential oil

Phytochemicals	Terpenoids	Alkaloids	Glycosides	Flavonoids	Tannins	Saponins	Steroids	Phenols
	+	+	+	+	-	+	+	-

Key: (+), present (-), absent

3.4 Antioxidant Activity

Antioxidant activity of essential oil of *Citrus* fruit peels has been tested by DPPH radical scavenging activity taking ascorbic acid as standard. The essential oil showed lower antioxidant potential as compared to ascorbic acid as shown in Fig. 2. From the results, it was revealed that the radical scavenging activity of oil was concentration dependent and increased with increase in concentration. These values are in agreement with as reported in the literature [31]. The antioxidant properties of *Citrus* fruit peel essential oil are mainly due to the presence of monoterpene, sesquiterpene hydrocarbons and their derivatives such as limonene (57.57%) whose antioxidant properties have been reported in the literature [32,33,34]. Limonene showed antioxidative properties even at a lower concentration of the order of 10-50 µg/ml [35]. Limonene which was a major component in *Wedelia prostrate* along with alpha-pinene

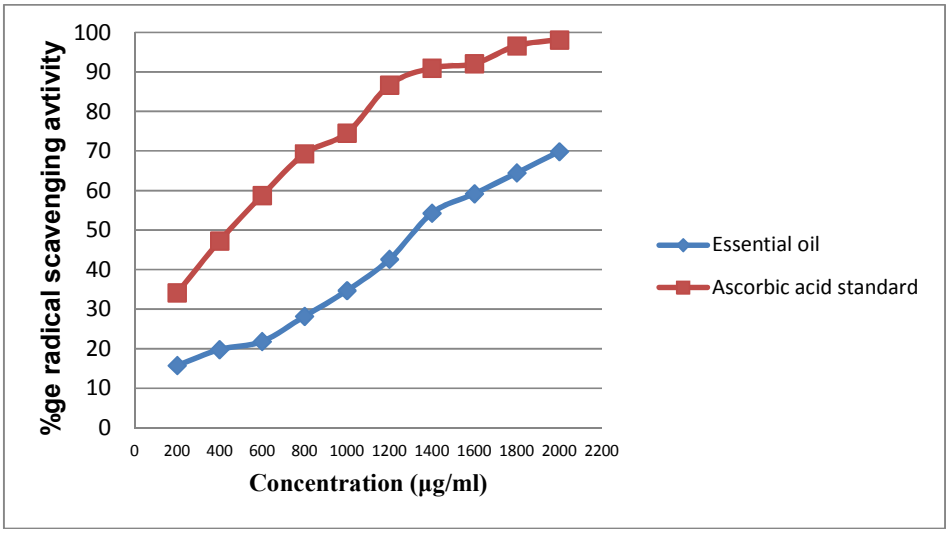

Fig. 2. DPPH radical scavenging activity of essential oil and ascorbic acid as standard

showed 88.15% DPPH radical scavenging activity [36]. The antioxidant potential of *citrus* peel essential oil vary depending upon the chemical composition and is mainly attributed to its major constituent, but the antagonistic or synergistic effect of various components in the mixture has to be taken into account [37].

4. CONCLUSION

From the above discussion, it is concluded that *Citrus reticulata* peel is a good source of different phytochemicals having medicinal properties and various dietary nutrients such as fibers, sugars and proteins. Hence it can be used as a novel dietary source of these nutrients. *Citrus* peel essential oil also has free radicals scavenging activity which was mainly due to the presence of various monoterpenes, sesquiterpenes and their derivatives. So intake of *Citrus* peel may reduce the risk of many oxidation related problems. Hence *Citrus* peel essential oil can be used as a natural antioxidant as a substitute for synthetic antioxidants which may be toxic or carcinogenic.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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