BJNS
Brazilian Journal of Natural Sciences

Brazilian Journal of Natural Sciences
www.bjns.com.br

ISSN 2595-0584 - V.3 N.2
July 2020 - pag. 354 - 364
Electronic journal

Review Article

A COMPREHENSIVE REVIEW ON CITRUS AURANTIFOLIA ESSENTIAL OIL: ITS PHYTOCHEMISTRY AND PHARMACOLOGICAL ASPECTS

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Article ID

Key words:
Citrus essential oil, monoterpenes, limonene, antifungal, antioxidant.

Abstract

Citrus essential oil, commonly, known as lime oil, has been widely reported in traditional system of medicine. Industrially, oil is isolated by mainly by hydrodistillation from fruit and peel of *Citrus aurantifolia*, family, Rutaceae. Cultivation practice of citrus plants dates back for over 4000 years and are one of most valuable fruit crops in the world. In this review, we aim to summarise the phytochemical and biological properties of citrus oil. The literature was collected from various online resources such as e journals, books and magazines. The citrus essential oil is globally used in food industry to impart citric flavour and odour to cuisines. Llime juice and oil is known to possess multiple biological properties including anti-cancer, antimicrobial, antioxidant, antiulcer, anti-inflammatory, hypolipidemic, antityphoid and hepatoprotective properties. Due to potent antibacterial and antifungal properties, citrus oil is becoming important component of skin care products. The medicinal importance of plant is due to presence of various secondary metabolites, alkaloids, carotenoids, coumarins, essential oils, flavonoids, phenolic acids, and triterpenoids. The citrus oil is rich in aromatic compounds namely, monoterpenes and

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DOI: https://doi.org/10.31415/bjns.v3i2.101 - Article received on July 01 , 2020; Accepted on July 07, 2020; published on 23 July 2020 in the Brazilian Journal of Natural Sciences, Vol. 3, N.2, online, ISSN 2595–0584. www.bjns.com.br. All authors contributed equally to the article. The authors declare that there is no conflict of interest.

their derivatives, aldehydes, ketones, esters, alcohols such as limonene (58.4%), β-pinene (15.4%), γ-terpinene (8.5%), citral (4.4%) and others. The bitter taste and aroma of citrus fruit peels is attributed to limonoids. *p*-caryophyllene constitute 5.7% of all the sesquiterpenes. On the basis of the available information, we conclude that citrus oil possess huge potential to be developed into pharmaceutical products.

Introduction

The genus Citrus (Rutaceae) is one of the most widely consumed and economic important group (1). The global production of citrus fruits has significantly increased to 82 million tons in the years 2009–2010 (2). Around 70% of the world's total marketable citrus are grown in the America, Brazil, Mediterranean countries. Of these, India is the world's largest producer of different varities of limes (Table 1) while China produces most of the world's mandarins an important variety of lime (3,4). Citrus products are a rich source of vitamins, minerals and dietary fibers that are essential for growth and development of body. The fruits possess anti-cancer (5), antimicrobial, antioxidant (6), antiulcer, anti-inflammatory, and hypolipidemic, antityphoid and hepatoprotective (7) properties. The essential oils contain many volatile compounds, mainly aldehydes, ketones, esters, alcohols and terpenes, which give the characteristic aromas and flavours of the citrus fruits. Because of their nutritional values and pleasant aromas, some of citrus fruit juices are used as functional drink (3, 8). Limonoids are the principal compounds found in citrus fruit peels where they produce the bitter taste and the zest aroma (9–11). Citrus-peel essential oils are amongst the most important of these, including orange, lemon, mandarin, tangerine and grapefruit oils are leading oils in terms of volume (12,13).

Table 1. Some popular varieties of citrus

S. No.	Variety	Reference
1	Sweet oranges (*Citrus sinensis* Osbeck)	(14)
2	Mandarins (*Citrus reticulata* Blanco)	(15,16)
3	Grapefruits (*Citrus paradisi* Macfadyen)	(17)
4	Lemons (*Citrus limon* Burmann)	(18)
5	Limes (*Citrus aurantifolia* Swingle)	(19)

Chemical constituents

The peculiar phytochemical composition of the peel and leaf oils of *C. aurantifolia* suggest use of the essential oils as a characteristic taxonomic marker for species (20) (Table 2, Fig. 1). The phytochemistry of citrus oil has been studied extensively by many researchers. GC-FID and GC-MS of hydrodistilled essential oil of *C. aurantifolia,* shows presence of limonene (58.4%), *β*- pinene (15.4%), β-terpinene (8.5%), and citral (4.4%) as the major constituents (21). Some exclusive terpenes such as the sesquiterpene santal-10-en-2-ol have been identified in the lime peel oil (22). An oxygenated monoterpene, fenchol, has also been isolated in *C. aurantifolia* (23,24). Some other mono- and sesquiterpene hydrocarbons and oxygenated monoterpenes such as *β*-pinene, neryl acetate, geranyl acetate, *β*-bisabolene, (*E*)-*α*-bergamotene, germacrene D and *β*-caryophyllene (25) have also been reported in *C. aurantifolia.* In addition, lime oil also contain coumarins which are known to cause phototoxic reaction in humans. In experimental animals, these coumarins were found to promote tumour formation on skin and abdominal epithelium of mice induced by 9,10-dimethyl-1,2-benxanthracene and benzo-[a]-pyrene (26–28).

C. aurantifolia peel oil

The chemical composition of *C. aurantifolia* peel oil is very similar to that of *C. hystrix*, a Malaysian citrus species with presence of monoterpenes (94.6%). The two most abundant compounds were limonene (39.3%) and *p*-pinene (28.4%). However, the former can be distinguished by the presence of relatively high concentrations of geraniol (7.5%), neral (5.3%) and geranial (2.1%), citronellal (0.1%) with absolute absence of citronellol. GC-MS analysis of some species of citrus, *C. hystrix* D.C., *C. aurantifolia* Swingle, *C. maxim* Merr. and *C. microcarpa* Bunge, revealed that *C. hystrix* peel oil comprises mainly of monoterpenes (97.2%) with *p*-pinene (39.3%), limonene (14.2%), citronellal (11.7%) and terpinen-4-ol (8.9%) as the major components. Other monoterpenes present in appreciable amounts include α-terpineol (3.0%), terpinene (2.4%), α-pinene (2.0%), linalool (l.9%) and furanoid cis-linalool oxide (1.9%). 17 sesquiterpenoids in smsll quantities constituting 2.6% of the oil have also been identified in the lime essential oil. Myrcene, is present at 1.6% and 1.8% concentrations in the peel oils of *C. maxima* and *C. microcarpa*. In comparison, peel oils of *C. maxima* and *C. microcarpa* contained more than 94% of monoterpene hydrocarbon, limonene, and could be one of the important natural sources of limonene.

C. aurantifolia leaf oil

The leaf oil of *C. aurantifolia* contain the highest concentration of monoterpenes amongst other species of citrus. Geranial (19.4%), limonene (16.4%), neral (11.4%), nerol (9.5%), geraniol (7.5%) and geranyl acetate (6.6%) are the major constituents of the leaf oil of *C. aurantifolia*. Sesquiterpenes present in amounts greater than 1% concentration are *p*-caryophyllene (5.7%), (*Z*)-nerolidol (2.0%), (*Z*)-*p*-farnesene (1.8%) and *p*-elemene (1.6%). whilst, the leaf oil of *C. hystrix* contains mainly citronellal (72.4%) and related compounds, citronellol (6.7%) and citronellyl acetate (4.1%). Of the other 39 components present in the leaf oil of *C. hystrix,* only *p*-pinene (1.9%), linalool (l.7%) and trans-sabinene hydrate (1.5%) are present at greater than 1% concentration. Sesquiterpenes accounts for only 4.5% of the oil. In contrast, *C. macrocarpa* leaf oil possess more than 70.8% sesquiterpenes with hedycaryol (19.0%), *p*-sesquiphellandrene (18.3%), α-eudesmol (l4.4%) and *p*-eudesmol (8.6%). While, *p*-pinene (13.4%), linalool (6.1%) and (E)-*p*-ocimene (2.0%) are main the monoterpenes *C. macrocarpa* leaf.

Table 2. List of phytochemicals in *C. aurantifolia*

S. No.	Type	Compounds	Reference
1	Sugars	Glucose, fructose and sucrose (1-15%)	(10,29,30)
2	Polysaccharides	Cellulose, hemicelluloses and pectin	(31)
3	Organic acids	Citric and malic acids with small quantities of succinic, malonic, lactic, oxalic, phosphoric, tartaric, adipic and isocitric acids	(20,32)
4	Lipids	Phospholipids (0.1%), palmitic, palmitoleic, oleic, linoleic and linolenic acids	(14,33)
5	vitamins	Ascorbic acid, thiamine, riboflavin, niacin, pantothenic acid, inositol, biotin, vitamin A, vitamin K, pyridoxine, paminobenzoic acid, choline and folic acid	(33)
6	Inorganic elements	Potassium and nitrogen (80%), calcium, iron, phosphorus, magnesium and chlorine	(34)
7	Flavonoids	Flavanones, flavones and anthocyanins	(1,31,35)
8	Limonoids	Limonene	(36,37)
9	Volatile compound	Limonene	(21,38)

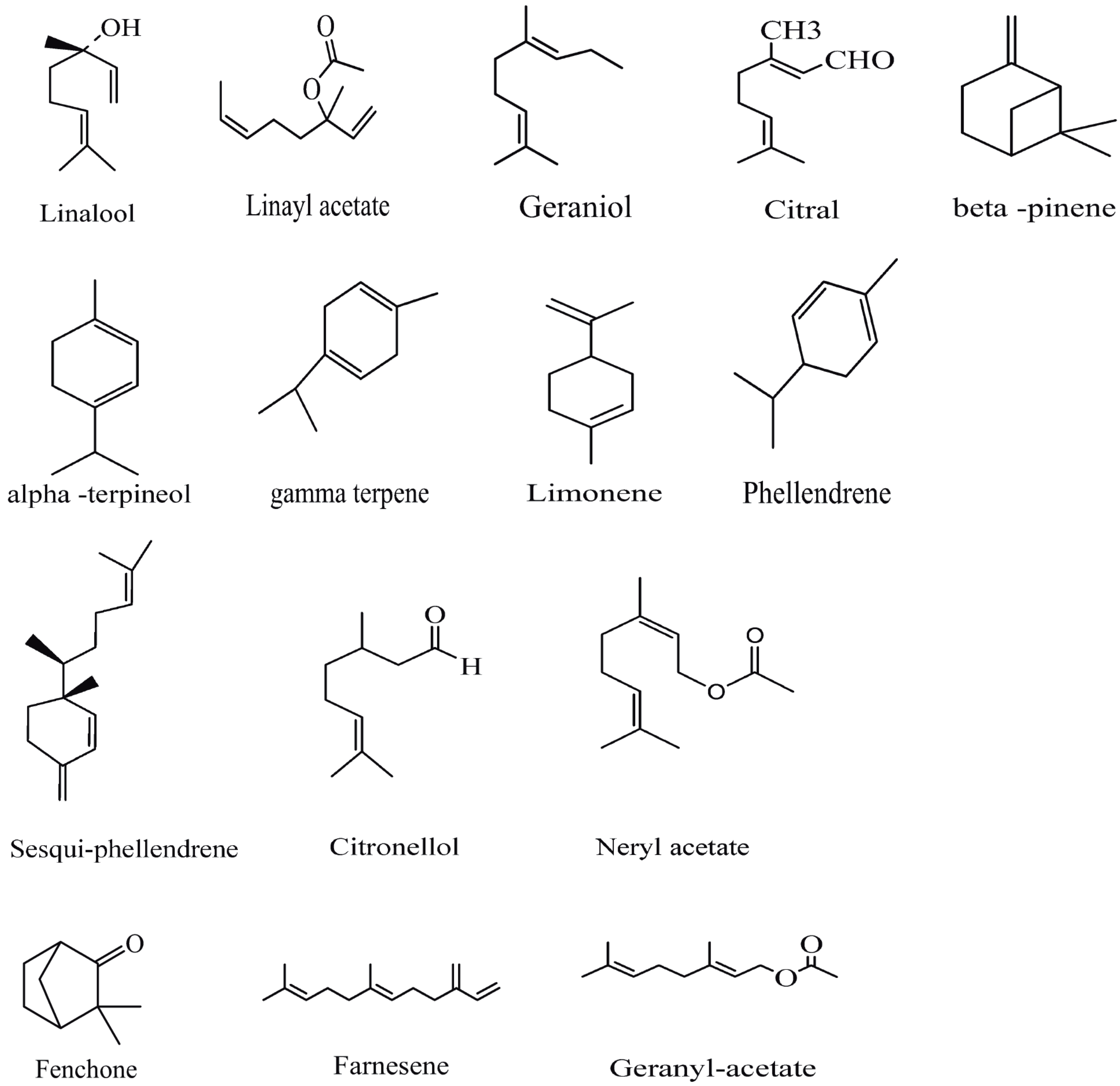

Fig. 1. Structures of major chemical constituents present in *C. aurantifolia* essential oil

Health Benefits of phytochemicals isolated from Citrus

Citrus is rich in flavonoids including apigenin, rutin, quercetin, kaempferol, nobiletin, hesperidin, hesperitin, and neohesperidin. Quercetin, has been reported as one of the most active flavonoids that possess significant anti-inflammatory, anti-tumor, anticancer, anti-prostatitis, anti-allergic and anti-asthmatic (31,39-43). Carotenoids found in citrus are β-carotene, lutein, zeaxanthin and cryptoxanthin (44,45). Presence of vitamin C in citrus enhances its medical applicability in treatment of stress, cold, chills, muscle fatigue and scurvy (40, 46–50).

Pharmacological activities of *C. aurantifolia*

Pharmacological activities of the extract of different parts of *C. aurantifolia* have been studied. The plant possesses the numerous biological activities described below:

Antibacterial activity

Antimicrobial activity of citrus oil against several pathogens, including, *S. aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas* spp, *A. niger* and *C. albicans,* has extensively been studied (28,51–55). Hydrodistilled lime oil (12.25-100 μg/ml) possess potent antibacterial activity against gram positive compared to gram negative strains (56). The values of zone of inhibition (ZOI) recorded for lime essential oil against some microbes investigated in study were, *S. aureus* (10 to 20 mm), *Enterococcus faecalis* (26 mm), *Salmonella* spp. (6-10 mm) and *C. albicans* (24 mm). The oil demonstrates powerful results in isoniazid-resistant strain of Mycobacteria that suggest probably, oil could have role in overcoming antimicrobial resistance (57,58). The antibacterial activity of *C. aurantifolia* has been attributed to the presence of phytochemicals, 5, 8-dimethoxypsoralen, 5-geranyloxypsoralen, palmitic acid, linoleic acid, oleic acid, 4-hexan-3-one and citral (58).

Abubakar U Zage found that citrus ethanolic extract (2.125-20 mg/ml) shows significant activity against clinical isolates of *Shigella, Salmonella typhi, Klebsiella*. The study indicated that Shigella was more sensitive to the extract with average zone of inhibition of 14.90 mm, followed by Klebsiella (14.49 mm), *E. coli* (13.77 mm) and *S. typhi* (12.01 mm) (59–61). In comparison, citrus peel methanolic extract is potent against *S. aureus* at concentration 31.25 µg/ml, while ethyl acetate extract is effective at higher concentration, 250-750 µg/ml (62).

Antifungal

The oil is becoming an important component of dermatological formulations used in skin and scalp diseases (63). Antifungal effects of citrus oil has been studied against *Malassezia furfur* in *in vitro* model using disk diffusion method. In a study, oil elicited fungistatic effects in a dose dependent manner with zone of inhibition of 2.6 mm at minimum inhibitory concentration (MIC) 2 mg/ml when compared to oil-untreated culture (64,65). Lime essential oil at 2 mg/ml was found to inhibit growth of *M. furfur,* KCCM 12679 cultured on sabrouds dextrose agar media with incubation temperature 37°C for 2-7 days, ZOI was found to be 2.6 mm when compared with reference standard (66).

In some studies, *A. niger* have shown explicitly high susceptibility to oil isolated from lime leaves (55). Matan. N. concluded that limonene in lime oil inhibits the growth of *A. niger* cultured in potato dextrose agar medium at 70°C. Additionally, monoterpene hydrocarbon, at MIC 90 µl/ml, also showed synergistic activity with other secondary metabolites present in lime oil (67, 68). Due to antifungal activity of citrus, the plant may be a potential candidate for use in agriculture and food industry for protection against aflatoxin contamination. The effects of lime essential oil on some species of molds has been determined by Matan and Matan. At concentration, 20–200 µl/ml, the oil was found to be fungistatic as well as fungicidal on the test species. Lime oil was effective at 100 µl/ml against *P. chrysogenum* and *Penicillium* sp. while *A. niger* was susceptibile at only higher concentrations of lime oil (140 µl/ml). The MIC values performed by the broth dilution of all conditions were examined (70).

Anti-obesity activity

Co-administration of *C. aurantifolia* essential oil with ketotifen in wistar mice suppressed weight gain in animals. The weight loss was described due to possibility of promoting anorexia, reduction in both the amount of food intake compared with the control group. (71)

Anticancer/cytotoxic activity

C. aurantifolia fruit from Texas, USA, consists of at least 22 volatile compounds, and its major compounds limonene (30%) and dihydrocarvone (31%) and five active components of *C. aurantifolia* seeds such as limonin, limonexic acid, isolimonexic acid, β-sitosterol glucoside, and limonin glucoside. Patil and group reported that 100 µg/ml extract of *C. aurantifolia* inhibits the growth of colon SW-480 cancer cell in 78% after 48 h of exposure. It increased level of caspase-3. (72). They also reported that *C. aurantifolia* extract can stop the growth of pancreatic Panc-28 cancer cells with inhibitory concentration 50%, IC_{50}, 18–42 µM. Among all the phytochemical tested in study, the order of apoptosis was isolimonexic acid > limonexic acid > sitosterol glucoside > limonin > limonin glucoside, based on the expression ratio of Bax/Bcl-2) (73).

Antioxidant property

Both fruit and peel juice of *Citrus aurantifolia* posses numerous flavonoids that contribute to antioxidant effects of plant. Lime juice and peel inhibits LDL oxidation in a dose dependent manner. At 234 nm polyphenolic extract solution of fresh lime juice (0-40 µl) prepared in DMSO, showed significant antioxidant property measured by lowry method (74). Patil and group revealed that freeze-dried lime juice extracted with different solvents, such as chloroform, acetone, methanol and methanol/water (8:2). The chloroform extract showed the highest (85.4 and 90%) radical-scavenging activity analysed by 1,1-diphenyl-2-picryl hydrazyl (DPPH) and 2,20 -azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) methods at 624 µg/ml (72). Limonoids possess the ability to inhibit tumor formation by stimulating the enzyme glutathione S-transferase (GST), enzyme that catalyzes the reaction of glutathione (75). Endogenously produced radical oxygen species (ROS) perpetuate ongoing inflammation that is a major factor in airway remodelling in asthma. Vitamin C is a major antioxidant present in airways. The plants enriched with phenolics and ascorbic acid have shown promising results in counteracting the radical production in lungs, thereby, indicating prophylactic role of plant in several diseases including, asthma (76-80). Concentrated juice of *C. aurantifolia* cv. swingle (Lime) at 250 µg/l, is able to significantly inhibit proliferation of phytohaemagglutinin activated mononuclear cells suggesting immunomodulating activity of plant that suggests immuno-modulatory property of plant (81, 82).

Anti-cholinesterase activity

The essential oils isolated from some species of *C. aurantifolia* and *C. aurantium* have shown significant inhibitory activity on AChE and BChE with IC50 values of 139.3-147.5 µg/ml and 235.5 to 266.6 µg/ml respectively (83, 84).

Anti-fertility activity

In experimental animals, oral administration of undiluted lime juice to Sprague-Dawley female rats has shown to compromise fertility by partially blocking ovulation, ova formation (5.10 +/- 2.37) in comparison with the control animals (12.70 +/- 1.14) (85).

Cardiovascular activity

C. aurantifolia is used in African folk medicine for the management of hypertension. the effect was validated in ex vivo studies conducted on isolated heart of rabbit. Aqueous extract of *C. aurantifolia* (10-80 mg/ml-10-20 mg/ml) produced both negative inotropic and chronotropic effects on the heart induced a dose-dependent relaxation of contractions produced by adrenalin (3.10-3 mM) and KCl (80 mM) (86). In cadmium induced hypertensive model of spargue daweley rats, *C. aurantifolia* fruit extract, 0.75 g/kg, was able to successfully reduce both diastolic and systolic blood pressure (87).

Conclusion

C. aurantifolia is valued for its nutritional qualities and numerous health benefits. The innumerable health benefits of *C. aurantifolia* and its essential oil are attributed to multitude of bioactive compounds including terpenes and phenolic components. This opens new horizons for development of essential oils into pharmaceutical products. However, lack of scientific evidence to confirm medicinal value warrants need of huge research in this direction.

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