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The Medicinal And Nutritional Role of Underutilized Citrus Fruit- *Citrus hystrix* (Kaffir Lime): A Review

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ABSTRACT

Citrus hystrix, commonly known as kaffir lime and medicinal lime as mentioned in ancient literature and belonging to *Rutaceae* family and it is native to everywhere within India and South East Asia. The fruits are green when raw and greenish yellow when fully ripe. They are used to prepare juice, pickles and as acidulant in curries. In the traditional Indian system of medicine the Ayurveda and various folk systems of medicine, the fruit peel and leaves are used to treat various inflammatory ailments. Chemical studies have shown that fruit contains various phytoconstituents like glycerolglycolipids, tannins, tocopherols, furanocoumarins and flavonoids and alkaloids. Preclinical studies have shown that some of its phytochemicals possess antibacterial, antifungal, anticancer, chemopreventive, antioxidant, anticholinesterase, cardio and hepatoprotective effects. The present paper deals with review of phytoconstituents and pharmacological action of underutilized *C. hystrix* fruit.

Key words: *Citrus hystrix*, underutilized fruits, phytochemistry, pharmacological activity, health benefits.

1. INTRODUCTION

Indigenous plant species are important sources of nutrition and traditional medicine to the local people in the rural areas, where the availability of the food is less or is unaffordable to the poor people.[1] *Citrus hystrix* DC belonging to the family *Rutaceae* is an underutilized tropical fruit, is a small and bushy tree, about 3-5 m tall, which grows all over India and South East regions of Asia, Southern China, Malaysia and Thailand.[2] *C. hystrix* belonging to the family *Rutaceae* is an underutilized tropical fruit of Thailand. It is an underexploited tree and is known as wild lime, medicinal lime and kaffir lime in English. They were bumpy, green, maturing to yellow skinned citrus fruit with a highly acidic flavour. A very thorny bush with aromatic leaves. The fruits are subglobose to oblate-globose shape, with a distinct nipple on the stem end. It has thick rind, knobby and wrinkled nature.[3] Kaffir lime is used as an herbal medicine to cure many diseases such as heart disease, dizziness and indigestion and also as physical nourishment.[4] It has folkloric reputation to be used in flu, fever, hypertension, abdominal pains and diarrhea on infants.[5] The fruit skin was traditionally used among the Malaysian folks for washing their hairs and other parts of the body and also the fruit juice is rubbed onto the skin to soften or mixed with bath water to control body odor.[6] Extracts from the skin as well as juice are used as an insecticide for washing the head and treating the feet to kill land leeches. The regular use of rubbing fresh leaves on the teeth and gum could aid in dental health. The oil from leaves and fruits are used as perfumery and medicinal preparation. Alcoholic and chloroform extracts of fruit peel possesses post-coital anti-fertility activity.[7] Crude acetone extract of root exhibits the activity against HIV-1 protease.[8] The fruits are used in traditional cuisines and remedies such as headaches and sore throats by Thai people.[9] The methanolic extract of leaves is known to inhibit the herpes virus[10] and also used as mosquito repellent.[11] The fruits are used as pickle as well as in cooking. The leaves are strongly aromatic and one or two leaves can be torn, chopped or shredded and used in soups and curries preparation and also added in butter milk during the summer time in order to delay or stop the lipid peroxidation due to the presence of associated bioactive compounds i.e. polyphenols and enhance the digestion system of stomach.

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2. Phytochemistry

The leaves contain two glyceroglycolipids (1, 2-di-*O*-α-linolenoyl-3-*O*-ß-galactopyranosyl-*sn*-glycerol (DLGG) and 1-*O*-α-linolenoyl-2-*O*-

palmitoyl-3-*O*-ß-galactopyranosyl-*sn*-glycerol (LPGG) [12] and a-tocopherol.[13] Leaves contain the volatile compounds such as citronellal, α-pinene, ß-pinene, sabinene, myrcene, trans-ocimene, γ-terpinene, ?-cymene, terpinolene, copaene, camphene, limonene, copene, linalool, ß-cubebene, isopulegol, caryophyllene, citronellyl acetate, citronellyl propionate citronellol, linalool, nerolidol, isopulegol, caryophyllene and δ-cadinene.[14,15,16] Forty one volatile constituents were identified from the leaf oil such as 2, 6-dimethyl-5heptenal and 2,6-dimetyl-5-heptenol and three new compounds were identified (ρ-menthan-8-ol (dihydro-a-terpineol), guaiol, and 4-ρ-menthene).[6] Fifty four free and thirty-nine glycosidically bound volatile compounds were isolated from kaffir lime leaves.[15,17,18] Two flavanone glycoside namely hesperidine and neohesperidine and one furanocoumarin namely oxypeucedanin hydrate were isolated from leaves[19] and also contains eriocitrin, neoeriocitrin, narirutin, naringin, didymin and myricetin.[20,21] Phenolic acids (vanillic acid, ρ-coumaric acid, sinapic acid, *m*-coumaric acid, benzoic acid, cinnamic acid) were identified from the ethanolic extract of leaves.[22]

Polar phenolic compounds such as aglycones and glycosides of flavonoids, didymin, eriocitrin. neohesperidin and neoeriocitrin were identified in Peel of kaffir lime.[23] Peel contains flavanones (naringin and hesperidin), polymethoxyflavones (sinensetin, nobiletin and tangeretin).[24,25] Peel contains essential oils such as ß-pinene, sabinene, terpinen-4-ol, pinene, terpineol, 1, 8-cineole, citronellal, limonene, ρ-menthan-8-ol (dihydro-a-terpineol), guaiol, and 4-ρ-menthene.[6,26,27] Four new citrunosides (1-4) was isolated from peel, compound 1 is a 1-O-isopropyl-6-O-ß-D-glucopyranosyl esters of 5", 9"-dimethyl-2", 8" -decadienoic acid. Compound 2-4 possess a 1-O-isopropryl- ß-D-glucopyranosyl and a dihydroxyprenyl furanocoumarin moiety conjugated to the 3-hydroxy-3-methylglutaric acid as diesters.[28] Peel is a good source of dietary fiber, containing total dietary fiber (82 g/100g), insoluble dietary fiber (54 g/100g) and soluble dietary fiber (28 g/100g).[25]

Limonoid namely limonin was isolated from root.[19] Two coumarins (hystrixarin (1) and hopeyhopin (2), a benzenoid derivatives (hystroxene-I (3) and quinolinone alkaloid (hystrolinone (4) were isolated from the crude acetone extract of root.[8]

3. PHARMACOLOGICAL ACTION

3.1. Antimicrobial activity

It is a well known fact that essential oils from Citrus spp., possessed antimicrobial effect such as antibacterial and antifungal activity.[29,30] So that the essential from citrus has used to improve the shelf life and safety of minimally processed fruits, skim milk and low-fat milk.[31] The essential oil and crude ethanolic extract of Kaffir lime peel showed greater antibacterial action against twenty sero types of Salmonella and five species of enterobacteria than the crude ethanolic extract of kaffir lime leaves.[32] Volatile oil from the Kaffir lime peel exhibit the antimicrobial activity against *B. subtilis*, *E. coli*, *S. typhimurium* and *S. aureus*.[33]

The crude extract of Kaffir lime oil from peel could inhibit the growth of *Candida albicans* which causing dandruff on the scalp. The Kaffir aromatic oil at 1% (v/v) could inhibit the growth of *C. albicans* and having more effective than positive control ketoconasole.[34] Essential oil (ß- citronellol, linalool and citronellol) from the leaf of Kaffir lime exhibited insecticidal properties against *Spodoptera litura* at 26.748 µL/g.[16] Makrut leaf oil (citronellal) and makrut oil (limonene, terpinene and a- terpineol) exhibited antibacterial properties by disc diffusion method against *Moraxella catarrhalis*, *Haemophilus influenza*, *S. pneumonia*, *S. aureus* and *Acinetobacter baumannii*.[35]

Supercritical fluid extract of *C. hystrix* (stem and bark) have the possibility to be applied as a constituent of cosmetic products and medicines because they showed highest antibacterial activity against on *Basillus subtilis*, moderate activity on *B. cereus* and *Staphylococcus epidermidis*, and weak activity against *S. aureus* and *Propionibacterum acnes* which are known to cause various type of skin infections.[36] At 5 and 10% concentration the Kaffir lime extract could inhibit the fungal spore germination of *Colletotrichum gloeosporioides* and *Fusarium* sp.,[37] Isolated coumarins (hystrixarin and hopeyhopin), benzenoid derivatives (hystroxene) and quinolinone alkaloid (hystrolinone) from the acetone extract of Kaffir lime root were found to exhibit antibacterial activity against *Acinetobacter baumannii* and *E. coli* with MIC values <3.125 and 3.125 µg/ml.[8]

Kaffir lime peel has the potential to act as a natural antimicrobial agent and showed activity against *B. cereus*, *Salmonella typhi* and *Staphylococcus aureus*.[38] The ethyl acetate extract from kaffir lime peel exhibited broad spectrum of inhibitory activity against Gram-positive bacteria, yeast and molds including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Saccharomyces cerevisiae* var. *sake* and *Aspergillus fumigatus* TISTR 3180. The major component such as limonene, citronellal sabinene and ß-pinene may contribute to the antimicrobial activity.[26]

Alcoholic extract of Kaffir lime peel has been exhibited the antibacterial activity against *S. aureus*, *B. cereus*, *Vibrio cholera* Ogawa and *V. parahemolyticus*.[39,40] The methanolic peel extract of *C. hystrix* fruit possess antibacterial activity on human pathogenic bacteria such as *S. aureus*, *S. typhi*, *E. coli*, *K. pneumonia* and *Proteus vulgaris*. The maximum inhibition zone was recorded against *S. aureus* and *S. typhi* an inhibition zone of 19 mm and 22 mm respectively.[41]

Anti-inflammatory activity

Essential oil from *C. hystrix* reported to exhibit anti-inflammatory activity against *P. acne* using 5-lipoxigenase inhibition assay. The major component such as d-limonene in the essential oil could contribute to the inhibitory activity and observed IC_{50} value of 0.05 µl/ml were compared to that of positive control nordihydroguaretic acid. D-limonene could inhibit the *P. acne* and reduce inflammation as well as reduce the post-acne scar formation and help to relieve acne blemishes.[42]

Methanolic extract of leaves tested to measure the anti-inflammatory activity by 12-O-tetradecanoyl-phorbol 13-acetate (TPA) induces

edema formation on ICR mouse ears. Two glycerolipids (1,2-di-O-α-linolenoyl-3-O-ß-galactopyranosyl-*sn*-glycerol (DLGG), 1-O- α-linllenoyl-2-O-palmitoyl-3-O- ß-galactopyranosyl- *sn*-glycerol (LPGG) exhibited higher activity (32 and 43%) than positive control indomethacin (19%).[12]

3.2. Anti tumor activity

Methonolic extract of leaves were evaluated for hepatocarcinogenic activity against 2-amino-3,8-dimethylimidazo (4,5-f) quinoxaline. *C. hystrix* exerts strong promotive potential on 2-amino-3,8-dimethylimidazo (4,5-f) quinoxaline induced hepatocarcinogenesis in the rat model. The presence of some active chemicals in the leaves of *C. hystrix* could significantly enhance the hepatocarcinogenic effect.[43]

Two glycerolipids (1,2-di-O-a-linolenoyl-3-O-ß-galactopyranosyl-*sn*-glycerol (DLGG), 1-O- a-linllenoyl-2-O-palmitoyl-3-O- ß-galactopyranosyl- *sn*-glycerol (LPGG)) from methanolic extract of leaves of *C. hystrix* were evaluated to inhibit the tumor promoting activity of 12-O-tetradecanoyl-phorbol 13-acetate in mouse skin with dimethylbenz (a) anthracene (DMBA) and 12-O-tetradecanoylphorbol 13- acetate. Both lipids were potent inhibitors of tumor promoter-induced Epstein-Barr virus (EBV) activation.[12]

3.3. Anticholinesterase activity

The isolated Citrusosides A-D and furanocoumarins compounds from the hexane and methanolic extract of peel reported to possess butyrylcholinesterase inhibitory properties and observed IC_{50} of 11.2 , 15.4 and 23 µM was comparable to that of the standard Galanthamine (3.2 µM). It is confirmed that the presence of dioxygenated geranyl chain in the compounds to be vital for the anticholinesterase activity.[28]

Two flavanone glycosides (hesperidin and neohesperidin) and furanocoumarin (oxypeucedanin hydrate) from butanolic and dichloromethane extract of leaves and limonoid (limonin) from the ethyl acetate extract of roots investigated to possess low to medium anticholinesterase inhibitory activity toward acetylcholinesterase and butyrylcholinesterase. The highest activity were reported to the compounds neohesperidine and oxypeucedanin with the IC_{50} values of 0.16 mM toward AChE 0.26 mM BChE was compared to that of the positive control serine.[19]

3.4. Antioxidant activity

Methanolic extract of leaves were evaluated for antioxidant potential. It exerts its oxidative stress by scavenging hydroxyl radicals and inhibiting lipid peroxidation that causes oxidative damage to liver cancer cell line HepG2 cells due to the presence of flavonol (myricetin) in the leaves (68.4 mg/100 g).[21] Ethanolic extract of leaves were screened for the ferric reducing antioxidant power, ß- carotene bleaching and oxygen radical absorbance capacity assay. The study reported that leaves extracts exhibited FRAP value (781 mM TE/g), ß- carotene bleaching activity (35.67%) and ORAC assay (10.51 mmol TE/g).[44] Supercritical carbon dioxide extraction of leaves was reported for the higher total phenolic content (128.9 mg GAE/g extract) than the solvent extraction and higher DPPH radical scavenging activity with the IC_{50} of 0.065-0.300 mg/ml.[22] Juice of Kaffir lime was reported to possessed high phenolic (490.47 mg GAE/ 100 ml of juice) and flavonoid, (22.25 mg hesperidine equivalent/100 ml) content and exhibited good antioxidant activity by DPPH and FRAPS methods. Juice showed the scavenging activity against DPPH radicals to have IC_{50} values of 35 mg/100 ml and FRAP value of 89.0 µmol Fe2+ equivalent/100 mL of juice.[45]

3.5. Inhibitors of Nitric oxide generation

Coumarins (bergamottin, oxypeucedanin and 5-[(6', 7;-dihydroxy-3', 7'-dimethyl-2-octenyl) oxy-]-psoralen) from the Methanolic extract of fruits were exhibited as inhibitors of lipopolysaccharide and interferon induced nitric oxide generation in RAW 264.7 cells. Among the three coumarins the inhibitory activity of bergamottin was higher than the others and it showed (IC_{50} value of 14 µM) which was comparable to that of a synthetic L-arginine analogue inhibitor of iNOS (N-(iminoethyl)-L-ornithine) (IC_{50} value of 7.9 µM). The bergamottin has the potential to inhibits the LPS/IFN-γ-triggered iNOS expression pathways and /or iNOS enzyme activity.[46]

CONCLUSIONS

Citrus, popularly known as food article is the unique source of various types of compounds having diverse structure. *C. hystrix* is a versatile, nutritious fruit with a great variety of uses. Here, an attempt was made to address phytochemistry and pharmacology of the underutilized fruit *C. hystrix*. It is quite evident from this review that *C. hystrix* an important medicinal herb which are being utilized in the field of Ayurveda, Siddha and other medical systems. *C. hystrix* contains a number of phytoconstituents, which are the key factors in the nutritional and medicinal value of this plant. Almost all parts of this plant such as leaf, fruit, seed and root are used to cure a variety of diseases. Crude extracts and phytochemicals isolated from various parts of *C. hystrix*, as reviewed here have been found to have many pharmacological activities such as antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer and antioxidant activity. The underutilized fruit of *C. hystrix* is a very important part of biodiversity and it's sustainably use for future generations. Quite a significant amount of work has been done on the biological activity and possible application of these compounds and hence extensive investigation on its pharmacology and clinical trials is needed to exploit their therapeutic utility to cure various diseases. The nutraceuticals perspectives of dietary fiber prepared from peel and pulp of *C. hystrix* fruit may be further exploited through *in vivo* studies for the wider utilization.

Conflicts of Interest

All authors have none to declare.

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