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# Citrus hystrix

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## Scientific Name

*Citrus hystrix* DC.

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## Synonyms

*Citrus auraria* Michel, *Citrus echinata* Saint-Lager, *Citrus hyalopulpa* Tanaka, *Citrus kerrii* (Swingle) Tanaka, *Citrus macroptera* Montrouzier var. *kerrii* Swingle, *Citrus papeda* Miquel, *Fortunella sagittifolia* F.M. Feng & P. I Mao, *Papeda rumphii* Hasskarl.

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## Family

Rutaceae

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## Common Names

Caffir Lime, Ichang Lime, Kaffir Lime, Leech Lime, Mauritius Papeda, Porcupine Orange, Porcupine Orange Lime, Rough Lemon, Thai Bai Makrut, Wart Lime, Wild Lime.

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## Vernacular Names

**Burmese:** Shauk Cho, Shouk-Pote, Shauk Nu, Shauk Waing;

**Chinese:** Ma Feng Gan, Mao Li Qiu Si Ku Cheng, Ma Feng Cheng, Ma Feng Mao Gan Fatt-Fung-

Kam (Cantonese), Thai-Ko-Kam (Hokkien/Minnan);

**Czech:** Kaffir Citrus;

**Danish:** Kaffir Lime;

**Dutch:** Indonesische Citroenboom, Kaffir Limoen, Djeroek Poeroet;

**Eastonian:** Kaffir Laimilehed;

**Finnish:** Kaffir Limetti;

**French:** Combava, Limettier Hérissé, Citron Combara;

**German:** Indische Zitrone, Indische Zitronenblätter, Indonesische Zitronenblätter, Kaffir Limette, Makrut Limette, Langdorniger Orangenbaum;

**Guam:** Limon Admelo;

**Hebrew:** Aley Kafir Laim;

**Hungarian:** Kaffercitrom, Kaffir Citrom És Levél;

**India:** Kolumichai (Tamil);

**Indonesia:** Juuk Purut (Bali), Jeruk Purut (Java), Jeruk Obat, Jerut Sambal, Limo Purut;

**Japanese:** Bai Makkuruu, Kobu Mikan, Moorishasu Papeda, Kafaa Raimu, Purutto, Kafiru Raimu, Kobu Mikan;

**Khmer:** Kraunch Soeuth, Slirk-Krote Sirk;

**Kiribati:** Te Remen;

**Laos:** Khi-Hout, Makgeehoot;

**Malaysia:** Limau Purut, Limau Hantu, Limau Suwangi;

**Palauan:** Debechel;

**Philippines:** Kabog, Kamuntai (Bikol), Kolison, Kolobot (Bisaya), Amongpong, Amontau, Kolo-Oi, Kopalian, Mayagarin (Cebu Bisaya), Kapitan (Ibanag), Kamugau, Kamukau, Kamulan, Kapitan (Iloko), Pinukpuk (Kalinga), Piris

(**Pangasingan**) Malatbas (**Sambali**), Muntai (**Subanum**), Daruga, Duroga (**Sulu**), Buyak, Buyog, Kabuan, Kabugau, Kobot, Kolobot, Kolong-Kolong (**Tagalog**);

**Reunion Island**: Combava (**French**);

**Russian**: Kaffir Laim, Kaforskii Laim;

**Samoan**: Tipolo Patupatu;

**Sri Lanka**: Kahpiri Dehi, Kudala Dehi, Odu Dehi (**Sinhalese**);

**Spanish**: Naranja Puerco-Espín, Hojas De Lima Cafre, Hoja De Lima Kaffir, Lima Kaffir;

**Swedish**: Kafirlime;

**Thailand**: Bai Makrut, Luuk Makrut, Luuk Makruut, Ma-Kruut, Magood, Magrood, Makrut, Makut, Som Makrud;

**Tongan**: Lemani, Moli Lemani;

**Tuvaluan**: Laim;

**Vietnam**: Chanh Kaffir, Chanh Sác, Trúc;

**Yapese**: Gurgur Gurgumimarech.

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## Origin/Distribution

The species is native to tropical southeast Asia, southern China and Malaysia. It has been introduced and cultivated elsewhere in the tropics and sub-tropics including in northern Australia.

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## Agroecology

The species thrives in a warm and wet climate. Trees are mildly frost hardy and grow best in areas that receive only short, mild frosts. It thrives in organic rich, fertile and well-drained acid loam soil and requires adequate watering during the dry seasons for good growth and fruiting.

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## Edible Plant Parts and Uses

The fruit and leaves are popular spice ingredient in Asian cooking. The leaves impart an aromatic, strong, unique and spicy flavour to many food dishes. The leaves can be used fresh or dried, and can be stored frozen. The leaves are widely used in Thai cuisine like *Tom yum gai* (Tom-yum chicken) and *tom yum kung* (Tom-yum prawns),

Lao cuisine and Cambodian cuisine for the savoury paste known as “*Krueng*”. In Javanese and Balinese cuisine the leaves are used to prepare *sayur assam* (salty or sour vegetables) and other spicy curry dishes of fish and chicken and also in *sate*. They are also used in Burmese and Malaysian cuisine like beef and chicken *Rendang* (Minangkabau dish comprising coconut milk and a range of spices that include ginger, galangal, turmeric leaf, lemon grass and chillies). The fruit is also used in making *sambal*, *acar* (vegetable salad pickled in vinegar dried chillies and ground peanuts) and *dalca* (mutton curry). The juice of the fruit is often used for souring dishes. It is also added to fish or meat in order to make them more tender and fragrant or to flavour grilled fish and grilled beef. The jus is also made into refreshing drinks.

The fruit juice is added to *sambal petis* (chilli sauce with shrimp) and also to *rojak* or *petjel* (savoury spicy sauce) in Indonesia. In Thailand, the fruit is used for seasoning and to prepare drinks teas such as *Citrus hystrix* flavonoid-rich sachet, which has been promoted to have great potential as a natural antioxidant health product. The peels are dried, preserved and candied. The zest of the fruit is commonly used in Creole cuisine and also used to impart flavour to rums in the French Reunion Island and Madagascar.

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## Botany

A small tree 3–6 m high, often not straight, crooked with glabrous and spiny branches. Leaves are alternate, unifoliolate, broadly ovate to ovate-oblong, 7.5–10 cm long, dark green on top, lighter on the bottom, very fragrant with long petiole expanded into prominent wings, 15 cm long by 5 cm wide (Plates 1, 2 and 3). Leaf and expanded petiole appear to be a single “pinched” leaf (Plates 1 and 2). Leaf base is cuneate, or rounded, apex obtuse or slightly acuminate or notched. Inflorescence is both axillary and terminal and is 3–5 flowered. Flowers are small, fragrant, white; calyx cuspidate 4-lobed, white with violet fringe; petals 4–5, ovate-oblong, yellowish white tinged with pink; stamens 24–30



**Plate 1** Kaffir lime flowers



**Plate 3** Puckered, warty fruit and winged petioles



**Plate 2** Pendulous fruiting limb of Kaffir lime



**Plate 4** Harvested kaffir lime and leaves on sale in local market



**Plate 5** Close up of verrucose, warty fruit rind of Kaffir lime

free (Plate 1). Fruit is large, verrucose, warty or bumpy, globose, ovoid to elliptic, green (Plates 2, 3, 4 and 5) turning yellowish-green when ripe, 5–7 cm diameter, rind thick, pulp yellowish, very acid and bitter. Seeds are numerous, ridged,

ovoid-oblong. 1.5–1.8 by 1–1.2 cm, mono-embryonic with white cotyledons.

## Nutritive/Medicinal Properties

The nutrient composition of the fruit contains per 100 g edible portion was reported as: water 88.6 g, protein 0.8 g, fat 0.6 g, carbohydrate 8.5 g, fibre 0.8 g, Ca 57 mg, P 2 mg, Fe 0.1 mg, K 172 mg, carotene 16 µg, vitamin A 3 µg, vitamin B 1 0.02 mg, vitamin B2 0.07 mg, and vitamin C 37 mg. (Tee et al. 1997).

The fruit is rich in calcium, vitamin C and potassium and also contain vitamin B1 and 2.

The percentage and concentration of total phenolics in *Citrus hystrix* cv Davao lemon leaf, peel and juice were reported by Berhow et al. (1998) as: leaf:- 25.8% (1.22 mg/g) flavone/flavonol, 27% (0.81 mg/g) flavanone, 8.7% psoralen, 20.2% coumarin (0.29 mg/g); juice: 6.3% (0.01 mg/g) flavone/flavonol, 63.5% (0.10 mg/g) flavanone, 25.2% (0.02 mg/g) coumarin.

The percentage and concentration of flavanones reported in *Citrus hystrix* cv Davao lemon by Berhow et al. (1998) were as follows: leaf:- didymin 50% (0.4 mg/g), hesperidin 50% (0.4 mg/g); juice: didymin 28% (traces), hesperidin 72% (0.1 mg/g). The percentage and concentration of flavone reported in *Citrus hystrix* cv Davao lemon by Berhow et al. (1998) were as follows: leaf: rutin 65% (0.8 mg/g).

*Citrus microcarpa*, *Citrus hystrix*, *Citrus medica* var. 1 and 2, and *Citrus suhuiensis* were found to contain high amounts of flavones, flavanones, and dihydrochalcone C- and/or O-glycosides (Roowi and Crozier 2011). Among the major compounds detected were apigenin-6,8-di-C-glucoside, apigenin-8-C-glucosyl-2''-O-rhamnoside, phloretin-3',5'-di-C-glucoside, diosmetin-7-O-rutinoside, hesperetin-7-O-neohesperidoside, and hesperetin-7-O-rutinoside. Most of the tropical citrus flavanones were neohesperidoside conjugates, which are responsible for imparting a bitter taste to the fruit.

In the peel oil and the juice of kaffir lime, 102 and 90 components were identified, respectively

(Motoki et al. 2005). The main components in the peel oil were:  $\beta$ -pinene (22.7%), limonene (17.3%), sabinene (11.9%), citronellal (7.8%), terpinen-4-ol (7.2%), citronellol (3.6%), and linalool (2.6%), while in the juice were  $\beta$ -pinene (35.6%), sabinene (7.0%), limonene (5.9%), terpinen-4-ol (19.7%),  $\gamma$ -terpinene (4.4%), and linalool (2.8%). In another analysis, twenty six (26) compounds were identified from the peel and thirty seven (37) compounds identified from the fruit oils (Safian et al. 2005). The major compounds contained in the fruit peel oil were  $\beta$ -pinene (27%), limonene (24.7%) and sabinene (13.8%) while  $\alpha$ -terpeneol (15.8%),  $\beta$ -pinene (15.1%) and limonene (9.1%). Ibrahim et al. (1995) reported the fruit peel oil to have the following major volatile constituents:  $\beta$ -pinene (39.25%), limonene (14.16%), citronellal (11.67%), terpinene-4-ol (8.89%),  $\alpha$ -terpeneol (3.03%), citronellol (2.96%),  $\gamma$ -terpinene (2.36%),  $\alpha$ -pinene (1.99%), *cis*-linalool oxide (1.85%),  $\delta$ -3-carene (1.44%), terpinolene (1.60%) and geraniol (0.68%).

*Citrus hystrix* leaf is rich in vitamin E. Among 62 edible tropical plants analysed for  $\alpha$ -tocopherol content *Citrus hystrix* leaves (398.3 mg/kg edible portion) ranked second behind *Sauropus androgynus* leaves (426.8 mg/kg) (Ching and Mohamed 2001). Thirty-eight constituents were identified in the leaf essential oil of Kaffir lime representing 89% of the essential oil (Waikedre et al. 2010). The oil was rich in monoterpenes (87%) with  $\beta$ -pinene as the major component (10%) and low in limonene (4.7%). The essential oil of *C. hystrix* was characterized by high contents of terpinen-4-ol (13.0%),  $\alpha$ -terpeneol (7.6%), 1,8-cineole (6.4%), and citronellol (6.0%). The oil was found inactive against bacteria. Twenty nine compounds were found in the essential oil of kaffir lime leaves (Loh et al. 2011).  $\beta$ -citronellal was the major compound amounting to 66.85% of total oil followed by  $\beta$ -citronellol (6.59%), 5,9-dimethyl-1-decanol (4.96%), unknown (4.75%), linalool (3.90%), methyl citronellate (1.90%), geranyl acetate (1.80%) and citronellol (1.76%). Other compounds included 3-undecanol (1.04%), 2-(2-hydroxy-2-propyl)-5-methylcyclohexanol (0.96%), 1,8-terpin (0.95%), iso-

pregol (0.70%), 4,8-dimethyl-1,7,nonadien-4-ol (0.60%), geraniol (0.42%), terpinene-4-ol (0.34%), *cis*-2,6,-dimethyl-2,6-octadiene (0.33%), (*E*)-furanoid linalool oxide (0.27%), 4-methyl-6-hepten-3-ol (0.26%), 2,6,-dimethyl-5-heptenal (0.24%), *cis*-linalool oxide (0.24%), sabinene (0.20%), isopulegol (0.18%), 2-methyl-7-oxabicyclo-heptane (0.13%),  $\alpha$ -terpineol (0.11%),  $\beta$ -myrcene (0.08%), (*E*)-2,5-dimethyl-1,6-octadiene (0.08%), tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran (0.05%), nerolidol (0.04%) and 3-hexen-1-ol (0.03%).

### Antioxidant Activity

Kaffir lime contains antioxidants and has very good potential to be explored as sources of natural antioxidants. Recent studies showed that ethanol concentration was the most significant factor affecting the TPC (total phenolic content) (Chan et al. 2009). The optimum extraction conditions of TPC from fruit peels were found to be ethanol concentration of 52.9%, extraction temperature of 48.3°C, and extraction time of 126.5 minutes. Under the optimised conditions, the experimental value for TPC was 1291.8 mg GAE/100 g DW, which reasonably close to the predicted value (1268.8 mg GAE/100 g DW). Studies reported that method of processing of kaffir lime can significantly affect the content of flavonoids and their TAC (total antioxidant capacity) values and thereby affect the antioxidative capacity (Butryee et al. 2009). Using three different assays: oxygen radical absorption capacity, ferric reducing/antioxidant power, and scavenging effect on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical, it was found that boiling decreased TAC values. The amount of total flavonoids calculated as aglycone equivalents of eight identified flavonoids (cyanidin, myricetin, peonidin, quercetin, luteolin, hesperetin, apigenin and isorhamnetin) was 1,129 (deep frying), 1,104 (fresh without processing) and 549 (boiling) mg/100 g freeze-dried weight (dry matter exclude fat). Hesperetin was the predominant flavonoid. The total phenolic content expressed as grams of gallic acid equivalents/100 g fresh

weight (excluding fat) was 2.0, 1.9 and 1.8 in fresh, deep fried and boiled samples, respectively. In another study it was found that the antioxidative activities of kaffir lime in descending order were leaves > peels > stems and all were much higher than that of the control but lower than pegaga (*Centella asiatica*) (Jaswir et al. 2004).

The yield of phenolics from *C. hystrix* leaves, total phenolic content (TPC) and DPPH-IC<sub>50</sub> obtained were 5.06%, 116.53 mg GAE/g extract and IC<sub>50</sub> of 0.063 mg/ml, respectively (Jamilah et al. 2011). Better inhibition and TPC were obtained using supercritical carbon dioxide extraction method whereas higher yield and phenolic acids were obtained in the ethanol extracts. Phenolic compounds identified were vanillic acid, *p*-coumaric acid, sinapic acid, *m*-coumaric acid, benzoic acid and cinnamic acid.

The antioxidative activity of the extracts in both DPPH radical scavenging activity assay and linoleic acid model system assays followed the decreasing order of: *Cucurma longa* > *Murraya koenigii* > *Citrus hystrix* > *Pandanus amaryllifolius* (Idris et al. 2008). 2,000 ppm was chosen as the optimum concentration to be used in deep frying experiment. Extracts of *Pandanus amaryllifolius* and *Citrus hystrix* exhibited protective activity towards RBD (refined, leached and deodorised) palm olein that was comparable to BHT (butylated hydroxytolouene) during frying. The extracts were useful in improving and also maintaining the sensory characteristics of French fries. The French fries treated with herb extracts were acceptable by panelists until day 5 of frying. The natural antioxidants significantly lowered the rate of oil oxidation during deep-fat frying and maintaining the quality of French fries. They exhibited excellent heat-stable antioxidant properties and presented good natural alternative to existing synthetic antioxidants for the food industry. Studies indicated that the ethanol extract of the *Citrus hystrix* peel exhibited antioxidant properties in frying oil as determined by measuring peroxide value (PV), *p*-anisidine value (AnV), totox value, iodine value (IV), percent free fatty acids, color and viscosity of the oil and therefore displayed potential as one of the new sources of natural antioxidants.

## Anticancer and Antiviral Activities

*Citrus hystrix* has anticancer and antiviral activities. Three known coumarins isolated from *Citrus hystrix* were found to be inhibitors of both lipopolysaccharide (LPS) and interferon- $\gamma$  (IFN- $\gamma$ )-induced nitric oxide (NO) generation in RAW 264.7 cells (Murakami et al. 1999). The inhibitory activity of bergamottin ( $IC_{50} = 14 \mu\text{M}$ ) was comparable to that of N-(iminoethyl)-L-ornithine (L-NIO) ( $IC_{50} = 7.9 \mu\text{M}$ ), whereas oxypeucedanin and 5-[(6', 7'-dihydroxy-3', 7'-dimethyl-2-octenyl)oxy]psoralen, structurally different from bergamottin only in their side-chain moieties, were notably less active. It was found that coumarins in Group (A) bearing an isoprenyl (IP) or a geranyl (GR) group, were highly active; Group (B) bearing an IP group cyclized to a coumarin ring, moderately active; group (C) bearing an IP group modified with hydroxyl group(s) and/or having other functional groups except for the IP were completely inactive. Cellular uptake studies suggested that coumarins in group C were inactive because of poor permeability to the cell membrane.

Ethyl acetate fraction of kaffir lime leaves exhibited the highest cytotoxicity against 4 leukemic cell lines with  $IC_{50}$  values of 19.0, 35.3, 21.8 and 19.8  $\mu\text{g/ml}$  against HL60, K562, Molt4 and U937 cell lines respectively (Chueahongthong et al. 2011). These were higher than those fractions from hexane, ethanol and butanol. The methanol fraction exerted no activity ( $IC_{50}$  value  $>100 \mu\text{g/ml}$ ). None of the fractions were cytotoxic on peripheral blood mononuclear cells.

Two glyceroglycolipids isolated from the leaves, namely 1,2-di-O- $\alpha$ -linolenoyl-3-O- $\beta$ -galactopyranosyl-sn-glycerol (DLGG, 1) and a mixture of two compounds, 1-O- $\alpha$ -linolenoyl-2-O-palmitoyl-3-O- $\beta$ -galactopyranosyl-sn-glycerol (2a) and its counterpart (2b) (LPGG, 2) were found to be potent inhibitors of tumour promoter-induced Epstein-Barr virus (EBV) activation (Murakami et al. 1995). The  $IC_{50}$  values of 1 and 2 were strikingly lower than those of representative cancer preventive agents such as  $\alpha$ -linolenic acid,  $\beta$ -carotene, or (-)-epigallocatechin gallate. Compound 1 exhibited anti-tumour-promoting activity even at a dose ten times lower

than that of  $\alpha$ -linolenic acid in a two-stage carcinogenesis experiment on ICR mouse skin with dimethylbenz[ $\alpha$ ]anthracene (DMBA) and 12-O-tetradecanoylphorbol 13-acetate (TPA). The inhibition of the arachidonic acid cascade may be involved in antitumour promotion since 1 inhibited TPA-induced edema formation in the antiinflammation test using ICR mouse ears. In another study, *C. hystrix* significantly enhanced 2-amino-3, 8-dimethylimidazo (4,5-f)quinoxaline-associated preneoplastic liver cell focus development while *Boesenbergia pandurata* and *Languas galanga* had borderline effects in F344 male rats (Tiwawech et al. 2000). The results suggested that *C. hystrix* as well as *Boesenbergia pandurata* and *Languas galanga* may contain agents augmenting the hepatocarcinogenicity of 2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline. In a separate study, the methanolic extract of *C. hystrix* leaves (flavonoids and tannins) inhibited the herpes virus (HSV-1) with an  $ED_{50}$  (concentration which inhibited viruses by 50%) of 91  $\mu\text{g/ml}$  and a  $CC_{50}$  (concentration which reduced cell proliferation by 50%) of 210  $\mu\text{g/ml}$  (Fortin et al. 2002). However the extract was not effective against the poliovirus type 2.

## Antifertility Activity

*Citrus hystrix* has antifertility effects. Thai scientists found that alcohol and chloroform extract of *Citrus hystrix* peels effectively inhibited implantation, produce abortion and slightly hasten labor time when it was given from day 2 to 5, day 8 to 12 and day 15 until labour, respectively (Piyachaturawat et al. 1985). At the same dose level which interrupted pregnancy, the extract did not affect the estrous cycle. Neither uterotrophic effects nor induction of vaginal cornification was observed when it was given to spayed rats. However, the extract enhanced the uterotrophic effect of estradiol when both were simultaneously given. Additionally, the extract stimulated uterine contractions observed in an in situ study. It was suggested that these two effects might have been responsible for the interruption of pregnancy associated with the extract.

### Antimicrobial Activity

*Citrus hystrix* also has antibacterial activity. The volatile oil obtained from leaf, fruit peel and juice inhibited bacterial growth, which could be ascribed to the presence of citronellal (Suri et al. 2002). All Gram negative bacteria were weakly inhibited by the volatile oil. The antibacterial property of methanolic extract of stem, seed, peel, leaf and callus treated with 2.0 mM phenylalanine was also studied. Most of the extracts showed stronger inhibition effect against Gram positive bacteria. In a preliminary study, kaffir oil extract showed higher antibacterial properties compared to the fresh extract (Chaisawadi et al., 2005). The extract was inhibitory to *Bacillus cereus* and *Staphylococcus aureus*. In another study, Thai scientists reported that citrus peels showed stronger antimicrobial activities than their essential oils obtained from hydrodistillation (Chanthaphon et al. 2008). The ethyl acetate extract of kaffir lime (*Citrus hystrix*) peel showed broad spectrum of inhibition against all Gram-positive bacteria, yeast and moulds including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Saccharomyces cerevisiae* var. *sake* and *Aspergillus fumigatus* TISTR 3180. It exhibited minimum inhibitory concentration (MIC) values of 0.28 and 0.56 mg/ml against *Saccharomyces cerevisiae* var. *sake* and *B. cereus*, respectively while the minimum bactericidal concentration (MBC) values against both microbes were 0.56 mg/ml. The MIC values of the extract against *L. monocytogenes*, *A. fumigatus* TISTR 3180 and *S. aureus* were 1.13 mg/ml while the MBC values against *L. monocytogenes* as well as *A. fumigatus* TISTR 3180 and *S. aureus* were 2.25 and 1.13 mg/ml, respectively. The major components of the ethyl acetate extract from kaffir lime were limonene (31.64%), citronellal (25.96%) and  $\beta$ -pinene (6.83%) whereas  $\beta$ -pinene (30.48%), sabinene (22.75%) and citronellal (15.66%) appeared to be major compounds of the essential oil obtained from hydrodistillation.

In a separate study, Kaffir lime oil was found to be inhibitory to *Propionibacterium acnes* which had a role in the inflammation of acne leading to scar formation (Lertsatitthanakorn

et al. 2006). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of kaffir lime oil were 5  $\mu$ l/ml. Antiinflammatory activity of the oils as determined using the 5-lipoxygenase inhibition assay found that  $IC_{50}$  value kaffir lime oil (0.05  $\mu$ l/ml) was less than that of nordihydroquarectic acid (1.7  $\mu$ g/ml).

### Cholinesterase Inhibitory Activity

Four new compounds, citrusosides A-D (1–4), and 15 known compounds were isolated from the hexanes and  $CH_2Cl_2$  extracts of *Citrus hystrix* fruit peels (Youkwan et al. 2010). Compound 1 was identified as the 1-O-isopropyl-6-O- $\beta$ -D-glucopyranosyl ester of 5'',9''-dimethyl-2'',8''-decadienoic acid. Compounds 2–4 possessed a 1-O-isopropyl- $\beta$ -D-glucopyranosyl and a dihydroxyprenyl furanocoumarin moiety conjugated to the 3-hydroxy-3-methylglutaric acid as diesters. Several furanocoumarins exhibited cholinesterase inhibitory activity. (R)-(+)-6'-hydroxy-7'-methoxybergamottin, (R)-(+)-6',7'-dihydroxybergamottin, and (+)-isoimparatorin showed  $IC_{50}$  values of 11.2, 15.4, and 23  $\mu$ M, respectively. Bioassay results indicated that the presence of a dioxygenated geranyl chain in the test compounds was crucial for the inhibitory activity.

### Skin Conditioning Activity

Studies in Thailand reported that using kaffir lime oil for body massage had a stimulating and activating effect (Hongratanaworakit and Buchbauer 2007). The oil caused a significant increase in blood pressure and a significant decrease in skin temperature. Regarding the behavioural parameters, subjects in the kaffir lime oil group rated themselves more alert, attentive, cheerful and vigorous than subjects in the control group. The findings lend support to its use in aromatherapy such as causing relief from depression and stress in humans. However, Kaffir lime had been reported to cause extensive

phytophotodermatitis in a hiker (Koh and Ong 1999). This was caused by the application of the juice as a folk remedy to ward off biting insects.

### Insecticidal Activity

Kaffir lime oil exhibited low mosquito repellency against *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*, compared to the volatile oils of turmeric, citronella and hairy basil (Tawatsin et al. 2001). Without vanillin, *Citrus hystrix* volatile oil provided repellency against *Aedes aegypti* for 1 h only. However, in the presence of vanillin, the volatile oil of *Citrus hystrix* had an extended repellency to *Aedes aegypti* for up to 3 h. *Citrus hystrix* essential oil showed good potential for being used as a cockroach repellent (Thavara et al. 2007). *Citrus hystrix* essential oil showed the best repellency over other candidate essential oils (*Boesenbergia rotunda*, *Curcuma longa*, *Litsea cubeba*, *Piper nigrum*, *Psidium guajava* and *Zingiber officinale*) and naphthalene. The essential oil exhibited complete repellency (100%) against *Periplaneta americana* and *Blattella germanica* and also showed the highest repellency (among the essential oils tested) of about 87.5% against *Neostylopyga rhombifolia* under laboratory conditions. In the field, *Citrus hystrix* essential oil formulated as a 20% active ingredient in ethanol and some additives provided satisfactory repellency of up to 86% reduction in cockroaches, mostly *P. americana* and *N. rhombifolia* with a residual effect lasting a week after treatment.

### Traditional Medicinal Uses

The juice and rinds of the kaffir lime are widely used in traditional Indonesian medicine like jamu and the fruit is sometimes referred to in Indonesia as *jeruk obat* (medicine citrus). The leaves can be used to treat stomach ache caused by dyspepsia and insect bites. In Peninsular Malaysia, the fruit and leaves have been used for washing hair; the fruit is halved and the grated rind is rubbed on the head or the whole fruit is boiled and used as

shampoo. The peel has also been used in a general tonic medicine “ubat jamu” and the fruit juice in ointment. The rind has also been prescribed for treatment for worms and headache.

### Other Uses

Oil is also extracted from the rind for use in cosmetics and beauty products.

Extracts from the fruit skin as well as juice are used as an insecticide for washing the head and treating the feet to kill land leeches and as a bleach to remove tough stains. The timber is used for tool handles and the juice may be used as an adjunct in dyeing with annatto (*Bixa orellana*).

The essential oil from kaffir lime leaves was found effective in killing larvae of tobacco army worm, *Spodoptera litura* with LD50 value of 26.748 ul/g (Loh et al. 2011). The essential oil also exhibited antifeedant properties resulting in severe growth inhibition of the insect.

### Comments

The plant is propagated from seeds and grafting.

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