

## Pharmacological Activities of *Citrus hystrix*

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### ABSTRACT

Kaffir lime (*Citrus hystrix*) is a bushy, small and bushy plant which is mainly used for its fruit and leaves as a seasoning for cooking. Kaffir lime (*Citrus hystrix*) belongs to the Rutaceae family. Internationally, kaffir lime (*Citrus hystrix*) is known as "kaffir lime". Kaffir lime (*Citrus hystrix*) grows in the tropics and originates from Southeast Asia, India, Malaysia and China and is widely used in the Netherlands, Germany, France, Italy and Spain. Kaffir lime (*Citrus hystrix*) is also very popular as a medicine which is important in treating various diseases. In this article, the discussion on kaffir lime (*Citrus hystrix*) is focused on its chemical composition and medical value, especially on its pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, antitumor, antilarvae, and antifertility as well as the use of kaffir lime (*Citrus hystrix*) as orthodox medicine and its applications traditional.

## INTRODUCTION

Herbal plants are a type of plant that has an important source of nutrition for the community, especially local communities in rural areas who do not yet have comprehensive modern medical facilities. One of the plants used is kaffir lime (*Citrus hystrix*). Kaffir lime (*Citrus hystrix*) is a bushy, small and bushy plant which is mainly used for its fruit and leaves as a seasoning for cooking. Kaffir lime (*Citrus hystrix*) belongs to the Rutaceae family. Internationally, kaffir lime (*Citrus hystrix*) is known as "kaffir lime". Kaffir lime (*Citrus hystrix*) grows in the tropics and originates from Southeast Asia, India, Malaysia and China and is widely used in the Netherlands, Germany, France, Italy and Spain (Krueger and Navarro, 2007).

Kaffir lime plants have a height of about 3-6 m which is not straight or crooked, branched and thorny. Kaffir lime leaves alternate, unifoliolate, broadly ovate or oblong ovate about 7.5-10 cm long, dark green on the upper surface of the leaf and lighter green on the underside. Kaffir lime leaves have a distinctive or fragrant smell with wide leaf stalks measuring 15 cm x 5 cm. Each leaf consists of 2 parts and looks like a double leaf. The widened leaves and petioles of kaffir lime leaves look like a single pinched leaf. The base of kaffir lime leaves is pointed or rounded with a blunt apex or slightly tapered or notched. Kaffir lime flowers are small, fragrant and white with 4 white petals and purple margins. The shape of the petals is oval, yellowish-white, slightly pink, consisting of 4-5 petals and 24-30 stamens. The kaffir lime fruit is green (turns yellow-green when ripe), large, elliptical or ovoid in shape, and warty with a diameter of 5-7 cm. Kaffir lime skin is thick with yellowish flesh and tastes sour and bitter and has wrinkles on the surface of the fruit skin. Kaffir lime seeds in one fruit are numerous, serrated and oval-ovoid with a diameter of 1-1.2 cm and are monoembryonic with white cotyledons (Lim, 2012).



Figure 1. Kaffir lime plant. A. Kaffir lime tree. B. Lime leaves. C. Kaffir lime fruit. D. Kaffir lime flower. E. Kaffir lime seeds

## LITERATURE REVIEW

### Chemical Composition

Agouillal et al (2017) found that the essential oil of fresh kaffir lime leaves extracted by steam distillation and the Liken-Nikerson extraction method contained 61% -73% citronellal as the main component, 10% -14%  $\alpha$ -citronellol and 5% limonene. % -7%. In addition, citronellal was 72.4%,  $\alpha$ -citronellol was 6.7%, citronellyl acetate was 4.1%,  $\alpha$ -pinene was 1.9% and limonene was 0.1% as a minority component of kaffir lime leaves ( Md Othman et al, 2016). Waikedre et al (2010) reported the presence of 38 compounds from kaffir lime leaves. Kaffir lime leaf essential oil has a high terpinen-4-ol content of 13%,  $\alpha$ -pinene of 10.9%,  $\alpha$ -terpineol of 7.6%, 1.8-cineole of 6.4%, citronellol of 6. %, p-cimene was 5.6% and limonene was 4.7%. Nor (1999) reported that kaffir lime peel from Malaysia contained 39.3%  $\alpha$ -pinene, 14.2% limonene, 11.7% citronellal and 8.9% terpinen-4-ol which were identified as the main components. Then, there is sabinene of 20.1% and  $\alpha$ -citronellol of 3.3%. Another study on the extraction of kaffir lime essential oil using an automatic steam distillation process with uncontrolled temperature was conducted by Kasuan et al (2013). The results showed that kaffir lime essential oil contained 31.22% sabinene, 32.96%  $\alpha$ -pinene, 20.68% limonene, 0.135% camphene, 1.735% myrcene, and 7.53% citronellal. Hongratanaworakit et al (2007) reported that kaffir lime peel essential oil consisted of monoterpene hydrocarbons namely limonene of 30.73%,  $\alpha$ -pinene of 18.76%, terpinene-4-ol of 10.63%,  $\alpha$ -terpineol of 8.35%,  $\alpha$ -terpinene 6.18% and terpinolene 4.33%. Recent research from Aumeeruddy-Elafi et al (2016) found that the main compounds of kaffir lime leaf essential oil are  $\alpha$ -pinene of 3.02%, limonene of 83.89%,  $\alpha$ -pinene of 0.78%,  $\alpha$ -myrcene of 0. .89% with traces of 0.21% methyl eugenol. In the study of Wungsintaweekul et al (2010), the skin and leaves of kaffir lime contained citronellal of about 23.85%, elementol of 6.59%,  $\delta$ -cadinene of 5.96%, geranylacetate of 5.12%,  $\alpha$ -terpineol of 5. .15%, L-linalool 4.22%,  $\alpha$ -pinene 1.82%, limonene 1.13% and  $\alpha$ -humulene 1.09%.

Abirami et al (2014) reported that kaffir lime peel contains  $8.66 \pm 0.14\%$  moisture, 46.55 g carbohydrates per 100 g,  $8.04 \pm 0.25$  g crude protein per 100 g,  $8.04 \pm 0.25$  g crude fiber of  $35.35 \pm 4.51$  g per 100 g, coarse ash of  $6.43 \pm 0.05$  g per 100 g, and crude fat of  $3.63 \pm 1.16$  g per 100 g. Then, kaffir lime pulp contains moisture of  $7.2 \pm 0.42\%$ , carbohydrates of 57.21 g per 100g, crude protein of  $8.23 \pm 0.21$  g per 100 g, crude fiber of  $24.19 \pm 1, 36$  g per 100 g, coarse ash of  $5.14 \pm 0.2$  g per 100 g, and crude fat of  $5.24 \pm 0.56$  g per 100 g. Meanwhile, kaffir lime peel fiber contains  $6.94 \pm 0.02\%$  moisture, 46.32 g carbohydrates per 100 g,  $7.83 \pm 0.22$  g crude protein per 100 g, 39.68 g crude fiber.  $\pm 2.19$  g per 100 g, coarse ash of  $3.17 \pm 0.15$  g per 100 g and crude fat of  $3 \pm 0.14$  g per 100 g. Butryee et al (2009) reported that freeze dried kaffir lime leaves contained 8.3% moisture and 2.24% fat in a sample of 89.46 g per 100 g, heated frozen kaffir lime leaves had a moisture content of 7.7% and 2.58% fat in a sample of 89.72 g per 100 g, and frozen dried kaffir lime leaves fried in deep-fat had a moisture content of 4.4% and fat of 43.77% in a sample of 51.83 g per 100 g. Then, fresh kaffir lime leaves had a moisture content of 56.6% and fat of 0.6% in a sample of 52.36 g per 100 g. Fresh kaffir lime leaves also contained total phenolic of  $2,023.95 \pm 107.07$  mg GAE per

100 g and heated kaffir lime leaves contained total phenolic of  $1,794.35 \pm 52.47$  mg GAE per 100 g. Fresh kaffir lime leaves contain various flavonoid components, namely cyanidine  $123 \pm 7$  mg per 100 g, myricetin  $179 \pm 18$  mg per 100 g, peonidin  $206 \pm 47$  mg per 100 g, quercetin  $43 \pm 9$  mg per 100 g, luteolin  $25 \pm 5$  mg per 100 g, hesperetin  $365 \pm 48$  mg per 100 g, apigenin  $50 \pm 30$  mg per 100 g, and isorhamnetin  $113 \pm 22$  mg per 100 g. Then, kaffir lime leaves that were heated contained cyanidine of  $50 \pm 6$  mg per 100 g, myricetin of  $89 \pm 1$  mg per 100 g, peonidin of  $119 \pm 22$  mg per 100 g, quercetin of  $13 \pm 3$  mg per 100 g, luteolin of  $11 \pm 4$  mg per 100 g, hesperetin  $187 \pm 33$  mg per 100 g, apigenin  $18 \pm 11$  mg per 100 g and isorhamnetin  $61 \pm 1$  mg per 100 g.

## METHODOLOGY

This research method is a literature review, namely a systematic, explicit and reproducible method for identifying, evaluating and synthesizing research works and ideas that have been produced by researchers and practitioners. Purpose of research literature review to obtain a theoretical basis that can support solving the problem being studied. The theory obtained is the first step for researchers to better understand the problems being studied correctly according to the scientific framework of thinking.

## RESEARCH RESULT AND DISCUSSION

### Pharmacological Activities

In general, kaffir lime (*Citrus hystrix*) contains an excellent source of chemical compounds and the presence of these compounds can be a potential pharmacological activity in kaffir lime (*Citrus hystrix*) with the following explanation:

#### 1. Antimicrobial Activity

The essential oil and ethanol extract of kaffir lime peel showed greater antibacterial activity against twenty sero species of *Salmonella* and five species of *Enterobacter* than the ethanol extract of kaffir lime leaves (Nanasombat and Lohasupthawee, 2005). Essential oil from kaffir lime peel shows antimicrobial activity against *B. subtilis*, *E. coli*, *S. typhimurium* and *S. aureus* (Srisukha et al, 2012). Essential oil from coarse kaffir lime peel extract can inhibit the growth of *Candida albicans* which causes dandruff on the scalp. Kaffir lime essential oil 1% (v/v) can inhibit the growth of *C. albicans* and is more effective than the positive control of ketoconazole (Prasart and Chonlada, 2005). The content of essential oils from kaffir lime leaves (such as limonene, terpinene, and  $\alpha$ -terpineol) has antibacterial properties using the disc diffusion method against *Moraxella catarrhalis*, *Haemophilus influenza*, *S. pneumoniae*, *S. aureus*, and *Acinetobacter baumannii* (Srisukha et al, 2012). Aqueous extracts of kaffir lime stems and bark have the potential to be used as ingredients for cosmetic products and medicines because they show the highest antibacterial activity against *Bacillus subtilis*, moderate antibacterial activity against *B. cereus* and *Staphylococcus epidermis* and weak antibacterial activity against *S. aureus* and *Propionibacterium acnes* which are known to cause various types of skin infections (Pyo and Oo, 2007).

Isolated coumarins (hystrixarin and hopeyhopin), namely benzenoid derivatives (hystroxene) and quinolinone alkaloids (hystrolinone) from kaffir lime root acetone extract were found to show antibacterial activity against *Acinetobacter baumannii* and *E. coli* with MIC values of  $<3.125\mu\text{g/ml}$  and  $3.125\mu\text{g/ml}$  respectively (Panthong et al, 2013). Kaffir lime peel has potential as a natural antimicrobial agent and shows antimicrobial activity against *B. cereus*, *Salmonella typhi* and *Staphylococcus aureus* (Chaisawadi et al, 2005). Ethyl acetate extract from kaffir lime peel showed broad inhibitory activity against Gram positive bacteria and yeast including *S. aureus*, *B. cereus*, *L. monocytogenes* and *S. cerevisiae*. The main components of kaffir lime compounds such as limonene, citronellal sabinene, and  $\beta$ -pinene can contribute to antimicrobial activity (Chanthaphon et al, 2008). Alcoholic extract of kaffir lime peel has shown antibacterial activity against *S. aureus*, *B. cereus*, *Vibrio cholera* and *V. parahemolyticus* (Chalermprachai et al, 1988; Temsiririrkul et al, 1994). Methanol extract of kaffir lime peel has antibacterial activity against human pathogenic bacteria such as *S. aureus*, *S. typhi*, *E.coli*, *K. pneumoniae* and *Proteus vulgaris*. The maximum inhibition zones recorded for *S. aureus* and *S. typhi* were 19 mm and 22 mm, respectively (Ajithkumar and Panneerselvan, 2012).

## 2. Anti-inflammatory activity

Essential oil from kaffir lime was reported to show anti-inflammatory activity against *P. acne* using 5-lipoxygenase inhibition assay. The main components such as d-limonene in kaffir lime essential oil can contribute to the inhibitory activity and observed  $\text{IC}_{50} = 0.05\mu\text{l/ml}$  compared to the positive control, namely nordihydroguaretic acid. D-limonene can inhibit *P. acne*, reduce inflammation, reduce the formation of acne scars and help remove acne blemishes (Lertsatitthanakorn et al, 1006). Kaffir lime leaf methanol extract was tested to measure anti-inflammatory activity by 12-O-tetradecanoyl-phorbol 13-acetate (TPA) inducing the formation of edema in the ears of ICR rats. Two glycerolipids (1,2-di-O- $\alpha$ -linolenoyl-3- $\beta$ -galactopyranoyl-sn-glycerol (DLGG) and 1-O-linlleenoyl-2-O-palmitoyl-3-O-galactopyranoyl-sn-glycerol (LPGG)) showed higher anti-inflammatory activity (ie 32% and 43%) compared to the positive control, namely indomethacin, which was 19% (Murakami et al, 1995).

## 3. Antioxidant Activity

Kaffir lime leaf methanol extract was evaluated for antioxidant potential. This provides oxidative stress by capturing hydroxyl radicals and inhibiting lipid peroxidation which causes oxidative damage to liver cancer cells (HepG2) due to the presence of flavonol (myricetin) in the leaves (68.4 mg per 100 g) filtered for iron-reducing antioxidant power, bleaching  $\beta$ -carotene and oxygen radical scavenging capacity assay. Studies report that kaffir lime leaf extract shows FRAP values (by 781 mM TE per g), bleaching activity of  $\beta$ -carotene (by 35.67%) and ORAC test (by 10.51 mmol TE per g) (Aziman et al, 2012 ). Kaffir lime leaf carbon dioxide extract was reported to have higher total phenolic content (128.9 mg GAE per g) than solvent extracts and higher DPPH radical scavenging activity with an  $\text{IC}_{50}$  of 0.065 mg/ml-0.3 mg/ml (Jamilah et al, 2011). Kaffir lime

juice was reported to have high phenolic content (490.47 mg GAE per 100 ml) and flavonoids (22.25 mg hesperidine equivalent per 100 ml) and showed good antioxidant activity by DPPH and FRAPS methods. Kaffir lime juice showed DPPH radical scavenging activity with IC<sub>50</sub> = 35 mg per 100 ml and FRAP = 89 mol Fe<sup>2+</sup> equivalent per 100 ml (Ghafar et al, 2012).

#### 4. Antitumor Activity

Kaffir lime leaf metonolic extract was evaluated for antitumor or hepatocarcinogenic activity against 2-amino-3,8-dimethylimidazo (4,5-f) quinoxaline. Kaffir lime provides a strong potential against 2-amino-3,8-dimethylimidazo (4,5-f) quinoxaline inducing hepatocarcinogenicity in a mouse model. The presence of active compounds in kaffir lime leaves can significantly enhance antitumor or hepatocarcinogenic effects (Tiwawech et al, 2000). Two glycolipids (1,2-di-O-linolenoyl-3-O-β-galactopyranosil-sn-glycerol (DLGG) and 1-O-α-linlilenoyl-2-O-palmitoil-3-O-galactopyranosil-sn- Glycerol (LPGG)) from kaffir lime leaf methanol extract was evaluated to inhibit tumor activity of 12-O-tetradecanoyl-phorbol 13-acetate in rat skin with dimethylbenz (a) anthracene (DMBA) and 12-O-tetradecanoyl phorbol 13 acetate. These two lipids are potent inhibitors of tumor-induced Epstein-Barr virus (EBV) activity (Murakami et al, 1995).

#### 5. Antilarval Activity

A study of the essential oil of fresh kaffir lime leaves against tobacco armyworm (*Spodopteralitura fabricus*) using a topical application bioassay on uniform weight second instar larvae showed significant antilarvae activity against armyworm larvae after 24 hours and 48 hours of treatment with IC<sub>50</sub> values respectively 29.25 µg/ml and 26.75 µg/ml. In addition, the weight growth of the larvae given kaffir lime essential oil was lower than the control treatment (Agouillal et al, 2017). In the study of Mya et al (2015), the ethanol extract of kaffir lime leaves was used to assess antilarvae activity against *A. aegypti*, which is the main vector of dengue fever (Jansen and Beebe, 2010). The results showed that high concentrations of kaffir lime leaf ethanol extract could be used to eradicate *A. aegypti*. Concentrations of 2.4%, 2.1%, 1.8%, 1.5% and 1.2% of the ethanol extract of kaffir lime leaves tested resulted in larval mortality of 99.5%, 85.5%, 62.5%, 26.5%, and 2% for 24 hours. Ansori et al (2015) tested kaffir lime leaf extract (methanol and n-hexane) with concentrations of 500 ppm, 1375 ppm, 2250 ppm, 3125 ppm and 4000 ppm against the three instar larvae of *A. aegypti*. The number of deaths was calculated after 24 hours of treatment and it was reported that the n-hexane extract of kaffir lime leaves was more toxic and an effective biolarvicidal with LC<sub>90</sub>=2885 ppm compared to the methanol extract which had LC<sub>90</sub>=3180 ppm. For antilarval activity against *A. aegypti*, kaffir lime leaf essential oil produced the best response for the contact test with 56.1% and non-contact with a release of 63.3% at 2.5% concentration. Meanwhile, kaffir lime peel essential oil produced the strongest response with a release of 46.5% at 2.5% concentration.

## 6. Antifertility Activity

Piyachaturawat et al (1985) investigated the effect of oral administration of alcohol extract and chloroform of kaffir lime peel on antifertility activity in pregnant adult female rats (Wistar) by oral administration at different gestation periods. Studies have shown an increase in the uterotrophic effect of estradiol when both extracts are given together. In addition, kaffir lime extract stimulates uterine contractions. Both of these effects may be responsible for the associated pregnancy disorders. Alcoholic extracts and kaffir lime chloroform were also found to be effective in inhibiting implantation, resulting in abortion, and slightly accelerating the time of delivery when given consecutively from the 2nd to the 5th day, the 8th to the 12th day and the 15th day until delivery.

Giving kaffir lime chloroform extract at a dose of 1 g/kg resulted in implantation inhibition of  $62.2 \pm 14.5\%$ . However, giving chloroform extract at a dose of 1 g/kg twice a day from the 8th to the 12th day can interfere with pregnancy by  $91.9 \pm 5.5\%$ . Meanwhile, kaffir lime alcohol extract produced the same effect of  $86.3 \pm 9.6\%$ . According to the antiimplantation effect, the chloroform extract of kaffir lime has higher abortion activity than the alcohol extract of kaffir lime (Piyachaturawat et al, 1985).

## CONCLUSIONS AND RECOMMENDATIONS

Kaffir lime (*Citrus hystrix*) is a bushy, small and bushy plant which is mainly used for its fruit and leaves as a seasoning for cooking. Kaffir lime (*Citrus hystrix*) belongs to the Rutaceae family. Internationally, kaffir lime (*Citrus hystrix*) is known as "kaffir lime". Kaffir lime (*Citrus hystrix*) grows in the tropics and originates from Southeast Asia, India, Malaysia and China and is widely used in the Netherlands, Germany, France, Italy and Spain. Kaffir lime (*Citrus hystrix*) is an important plant because of its chemical composition and pharmacological activity which plays a role in treating various diseases. Kaffir lime (*Citrus hystrix*) has health benefits and values such as antimicrobial, anti-inflammatory, antioxidant, antitumor, antilarvae, and antifertility. Overall, consumption and utilization of kaffir lime (*Citrus hystrix*) should be further supported. More in-depth research is needed on the potential of other pharmacological effects.

## ADVANCED RESEARCH

It is necessary to carry out qualitative and quantitative test studies on the content of secondary metabolites from kaffir lime plants (*Citrus hystrix*).

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