Indonesian Journal of Life Sciences Vol. 02 | Number 02 | September (2020)
http://journal.i3l.ac.id/ojs/index.php/IJLS/

REVIEW ARTICLE

Phytochemical Screening, Therapeutic Benefits, and Adverse Effects of *Citrus aurantifolia* - A Review

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ABSTRACT

Key lime or *Citrus aurantifolia* has been well known for its functions, either as food or medicine. This fruit has a greenish-yellow color with a sour and bitter taste and distributed initially from East Asia. This review summarizes the phytochemical screening, therapeutic effects, and adverse effects of key lime. Most parts of the plant contain phytochemicals, such as phenols, flavonoids, steroids, terpenoids, and alkaloids, with other varying compounds like saponin, tannin, anthraquinone, glycosides, and carbohydrates that may be due to the country where the plant is grown, or the extraction methods. Some studies have been conducted to evaluate the therapeutic effects of key lime, including antibacterial, antioxidant, anticancer or antitumor, anti-cholesterol, anti-larvae, anti-mosquito, antidiabetic, anti-inflammatory, and anticholinesterase. Key lime also may cause some adverse effects, such as phytophotodermatitis. It may lead to toxicity manifested in conditions such as edema, inflammation, or necrosis in some organs in the body, an increase in lymphocytes & liver enzymes, and a decrease in hemoglobin.

Keywords: *Citrus aurantifolia*; key lime; Mexican lime; phytochemicals; therapeutic effects; adverse effects

INTRODUCTION

Plants play a significant role in human lives, especially in food and medicine. Many drugs are found and derived from plants. All parts of the plants can be used for medicinal purposes, but mostly the part of the plant extracted as a drug is the fruits. Fruits are known to be a source of nutrients, and it is recommended to consume them daily to get physiological benefits for the body (Conner *et al.*, 2017).

Key lime (*Citrus aurantifolia*) is a well-known fruit that is usually used in beverages and food. It is also known as 'Mexican lime'. Its use in medicinal have long been known. Contrary to its common name, this fruit is distributed and cultivated in tropical and subtropical countries, not limited to only one country (CABI, 2019). Usually, key lime is known for its rich vitamin content and its antioxidant properties (CABI, 2019). In fact, studies about key lime have been done, with topics surrounding various pharmacological effects, including antimicrobial, anti-cholesterol, anti-inflammatory, insecticidal, and even anticancer properties (Jeffrey *et al.*, 2020; Cyndi *et al.*,

2016; Amorim *et al.*, 2016, Muniandy *et al.*, 2020; Zhao *et al.*, 2017; Şeker Karatoprak *et al.*, 2020). This review will discuss these pharmacological effects of key lime, along with its description and toxicity reports based on studies compiled from the last five years, to summarize the scientific information of key lime.

BOTANICAL DESCRIPTION

There are five subtypes of cultivated limes. Key lime is categorized as small-fruited acid limes. According to the CABI official datasheet (2019), below is the taxonomic tree of key lime.

Domain : Eukaryota
Kingdom : Plantae
Phylum : Spermatophyta
Subphylum : Angiospermae
Class : Dicotyledonae

MORPHOLOGY

The fruits of *C. aurantifolia* are greenish-yellow in color and about 2.5 to 5 cm in diameter. This is what characterizes it from other lime types, while other species like Persian lime (*C. latifolia*) and Makrut lime (*C. hystrix*) are at least 5 cm in diameter. It has a strong citrus aroma, high acidity, and acid content (up to 7-8% citric acid). The key lime tree is a small to medium-sized polyembryonic plant that has a tart and bitter taste. It is a thinly but densely and irregularly branched, perennial, evergreen tree, about 3 - 5 meters high. The twigs of this plant are stiff, short and have sharp spines throughout them. Meanwhile, the stem is unusually slender and branched, with sharp thorns and spines. The leaves arrangement is alternate, and the leaves themselves are about 4-8 x 2-5 cm in size, with crenulated margin, elliptic to oblong-ovate in shape, narrowly winged petioles. The flowers are small, with white-colored buds, with short axillary raceme inflorescences. The seeds are small, ovoid, plump, pale, smooth, with white embryos. The fruits are round, 3 - 5 cm in diameter, roughly 42 gram in weight with 1 mm peel, has a green to yellow color with thin skin, acidic, aromatic, and juicy in nature (Khan *et al.*, 2017; CABI, 2019; Al-Aamri *et al.*, 2018; Chi *et al.*, 2019).

DISTRIBUTION

The plant is believed to have originated from East Asian origin, particularly in northern Malaysia, or northern India, next to Myanmar, which was then brought to North Africa, Palestine, and to Mediterranean Europe by Arabs for trading (Khan *et al.*, 2017). Then, the fruit is cultivated in tropical and subtropical countries, because most limes are tolerant to drought and prefer warm temperatures to grow (Khan *et al.*, 2017).

PHYTOCHEMICAL SCREENING

According to studies found, the quantity of bioactive compounds in various parts of key lime varies depending on its geographic location and extraction methods. However, qualitative identification mostly showed similar classes of bioactive compounds. The qualitative testing of various phytochemicals from various studies were summarized in Table 1.

Table 1. The phytochemical compounds reported of *C.aurantifolia.*

Author	Region	Plant Part	Extraction Method	Phytochemicals									
				Phe	Fla	Ste	Ter	Sap	Tan	Alk	Ant	Gly	Car
Jeffrey *et al.*, 2020	Java, Indonesia	Fruit peel	MeOH & EA	+	+	+	+	+	+	+	N/A	N/A	N/A
Nata'ala *et al.*, 2018	Sokoto, Nigeria	Stem	Aq & EtOH	N/A	+	+	N/A	-	-	+	+	-	+
Oikeh *et al.*, 2015	Edo, Nigeria	Fruit juice	Freeze-dried	+	N/A	+	+	+	N/A	+	N/A	N/A	+
M` *et al.*, 2017	Shimoga, India	Fruit peel & pulp	70% EtOH	+	+	+	+	+	+	+	N/A	+	N/A
Namania *et al.*, 2018	Nakhal & Nizwa, Oman	Leaf	95% EtOH	N/A	+	+	N/A	-	N/A	+	N/A	N/A	+
Okunlola *et al.*, 2019	Minna, Nigeria	Leaf & bark	EtOH	+	+	+	+	+	+	+	+	+	N/A
		Stem		+	+	+	-	+	+	+	-	-	N/A
		Seed		-	-	+	+	-	-	+	-	-	N/A

MeOH: methanol; Aq: aqueous; EtOH: ethanol; Phe: phenolics; Fla: flavonoids; Ste: steroids; Ter: terpenoids; Sap: saponin; Tan: tannin; Alk: alkaloid; Ant: anthraquinone; Gly: glycosides; Car: carbohydrates; N/A: not available (not tested); +: present; -: absent

Not much research had been done on the seeds, as it only contained three types of phytochemicals, which are alkaloids, terpenes, and steroids (Okunlola *et al.*, 2019). Generally, key lime fruit peel, juice, leaves, and stems all contain phenols, flavonoids, steroids, terpenoids, and alkaloids, while the presence of saponin, tannin, anthraquinone, glycosides, and carbohydrates varies. Many reasons could cause this variation. The samples used for studies were mostly collected from various countries and others in various regions of a country, and this resulted in the variation of phytochemicals of key lime. Other phytochemical testings that are not reported in Table 1 included negative result for protein and amino acids in key lime leaves obtained from Oman (Namania *et al.*, 2018), positive result for reducing sugars in key lime fruit juice obtained from Nigeria (Oikeh *et al.*, 2015), and a positive result for carotenoids in both key lime fruit peel and pulp obtained from India (M` *et al.*, 2017).

Quantitative analysis of phytochemicals in key lime showed more diverse results, even in different regions of the same country. A study by Amorim *et al.* (2016) found that the essential oil of fruit peel obtained from hydrodistillation contained 29 compounds. Its major constituent was limonene (31.1%), followed by γ-terpinene (10.8%), geranial (9.6%), β-pinene (8.5%), neral (7.1%), and β-bisabolene (6.8%). However, in a study by Lemes *et al.* (2018) that used the same methods of hydrodistillation and same plant part of fruit peel, only 17 compounds were found, and its major constituent was limonene (77.5%), followed by myrcene (4.4%), which was not present in the former. It was also found that the polysaccharide content of key lime fruit

peel yielded 80.33%, including glucose, galactose, galacturonic acid, mannose, arabinose, and rhamnose, with some protein (4.36%) and uronic acid (10.56%) (Zhao *et al.*, 2017). The phytochemicals that were found in other parts were summarized in Table 2.

Table 2. Concentration of phenolics and flavonoids reported of *C.aurantifolia.*

Author	Region	Plant Part	Extraction Method	Phytochemical	
				Total phenolic	Total flavonoids
Patil *et al.*, 2009	Texas, America	Fruit juice	Chloroform	3.65 g of CnE/100g	579.08 mg/100g
			Acetone	0.69 g of CnE/100g	104.29 mg/100g
			MeOH	4.2 g of CnE/100g	386.24 mg/100g
M` *et al.*, 2017	Shimoga, India	Fruit peel	70% EtOH	46.65 µg of GAE/mg ext	36.49 µg of ClE/mg ext
		Fruit pulp		32.46 µg of GAE/mg ext	15.19 µg of ClE/mg ext
Namania *et al.*, 2018	Nakhal, Oman	Leaves	95% EtOH	96.55 µg of GAE/mg dry ext	64.2 µg of QE/mg dry ext
	Nizwa, Oman			322.57 µg of GAE/mg dry ext	41.38 µg of QE/mg dry ext

CnE: catechin equivalent, GAE: gallic acid equivalent, ext: extract, ClE: catechol equivalent, QE: quercetin equivalent

In a further investigation of the contents of flavonoid of key lime, it was found that key lime juice contains flavanones and flavonols (Patil *et al.*, 2009). The major flavanone from total flavonoids is hesperidin (58.66% w/w), didymin (3.46% w/w), and hesperetin (1.87% w/w). In comparison, the only flavonol present is rutin (36.67% w/w of total flavonoids) (Patil *et al.*, 2009). One phytochemical unique to lime is limonoids, which are classified as triterpenoid. A study which utilized HPLC analysis found that the chloroform extract of key lime juice contains the most phytochemicals among all the extracts, including isolimonexic acid, limonexic acid, limonin, and hesperidin, and one glucoside which is limonin glucoside (Patil *et al.*, 2009).

THERAPEUTIC EFFECTS

Antibacterial

Nowadays, the search for natural antibacterial compounds is of particular interest, especially in the era of pathogenic bacterial resistance towards synthetic antibacterial agents. Studies highlight *C. aurantifolia* leaves, peel, and fruit as a source of antibacterial compounds, each with different compositions of phytochemical compounds (Oikeh *et al.*, 2015; Lee *et al.*, 2018; Al-Aamri *et al.*, 2018). Based on research, the most commonly used method of extracting essential oils from leaves is by hydrodistillation to yield deionized water extract, in which the water will be boiled off, and essential oil was dried using anhydrous sodium sulfate (Oikeh *et al.*, 2015; Lee *et al.*, 2018; Al-Aamri *et al.*, 2018). Al-Aamri *et al.* (2018) reported a dose-dependent effect of *C. aurantifolia* leaf essential oil with a more pronounced effect against *Staphylococcus*

aureus than in *Escherichia coli* based on the size of the inhibition zone; this extract is classified as generally recognized as safe (GRAS) and can also be used as gastroprotective agent.

Chi *et al*. (2019) used both broth dilution and disk diffusion method, and both highlighted that *C. aurantifolia* leaf essential oil showed strong antibacterial effect against *Staphylococcus aureus, Bacillus cereus,* and *Salmonella typhi* strains, and showed weak effects against *Pseudomonas aeruginosa*. Lemes *et al.* (2018) used the broth dilution method and reported moderate antibacterial activity against *Streptococcus sobrinus, Streptococcus mitis, Streptococcus salivarius,* and *Streptococcus sanguinis* strains, and promising antibacterial activity against *Streptococcus mutans* and *Lactobacillus casei* based on MIC value.

A study by Okunlola *et al.* (2019) ground *C. aurantifolia* leaves and added directly to agar and broth to determine inhibitory activity and MIC & MBC value toward 10 identified pond water bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Salmonella spp*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Kluyvera ascorbate*, *Proteus myxofaciens*, and *Enterobacter aerogenes*). It reported inhibitory activity on a concentration of 0.3, 0.4, and 0.5 g/L after 24 hours of treatment. It also reported similar MIC and MBC values towards all ten bacteria strains except for *Shigella dysenteriae*.

For the peel, Lemes *et al.* (2018) used hydrodistillation method to extract the essential oil content and reported moderate antibacterial activity against *Streptococcus sobrinus, Streptococcus mitis, Streptococcus salivarius,* and *Streptococcus sanguinis* strains based on MIC value of 100 - 200 µg/mL, and promising antibacterial activity against *Streptococcus mutans* and *Lactobacillus casei* based on MIC value of 20 and 31.25 µg/mL. Meanwhile, Jeffrey *et al*. (2020) macerated fruit peel using methanol, in which the methanol extract was further fractionated with different solvents (ethyl acetate, n-hexane, and water) and showed that n-hexane subfraction resulted in the best inhibitory activity against *Streptococcus mutans* based on inhibitory zone area and MIC and MBC value of 0.19% and 0.39% respectively. In addition, it was also shown that n-hexane subfraction at concentration of 2% already showed an inhibition zone, while another subfractions did not show any inhibition activity.

For fruit extract, Oikeh *et al.* (2015) extracted the juice using a manual juice extractor, lyophilized and concentrated at 4^{o}C, and it reported a higher inhibitory effect against Gram-positive bacteria (*Staphylococcus aureus* & *Enterococcus faecalis*) as compared to Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella spp.*) based on the inhibition area. While based on broth dilution method, it showed the highest inhibitory and bactericidal activity towards *Staphylococcus aureus* and *Salmonella spp.* (MIC: 12.5 µg/mL; MBC: 50 µg/mL), and moderate inhibitory and bactericidal activity towards *Enterococcus faecalis, Pseudomonas aeruginosa*, and *Escherichia coli* (MIC: 25 µg/mL; MBC: 50 µg/mL); these values suggested that fruit concentrates were bacteriostatic at lower concentrations, and bactericidal at higher concentrations. Berlian, Fatiqin, and Agustina (2016) also processed the fruit of *C. aurantifolia* similarly, with additional dilution steps to determine effective concentration to inhibit *Escherichia coli* strains in food samples. The result showed that undiluted lime juice extract showed the best inhibitory activity towards

Escherichia coli and could be added to food samples and safely consumed.

Parama, Sukrama, and Handoko (2019) macerated the dried fruits in methanol and evaporated it to retrieve the extract, and the result reported a dose-dependent inhibition to *Streptococcus mutans* strains, with an overall significant inhibitory effect, and 80% concentration showed the most significant inhibitory effect.

Nata'ala *et al*. (2018) utilized the stem of *C. aurantifolia*, which was grounded and extracted, and reported the MIC value of 50 mg/ml ethanolic extract against *Staphylococcus aureus* and *Proteus mirabilis*, 100 mg/ml ethanolic extract against *Klebsiella pneumoniae*, and 100 mg/ml aqueous extract against *Klebsiella pneumoniae*, while the MBC value was reported to be 100 mg/ml ethanolic extract against *Staphylococcus aureus* and *Proteus mirabilis*, 200 mg/ml ethanolic extract against *Klebsiella pneumoniae*, and 200 mg/ml aqueous extract against *Klebsiella pneumonia.* Meanwhile, Okunlola *et al.* (2019) reported the inhibitory activity of plant seed, bark, and stem at concentrations of 0.3, 0.4, and 0.5 g/L after 24 hours' treatment, and also reported similar MIC and MBC value towards *Bacillus megatarium*, *Kluyvera ascorbate, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus myxofaciens, Klebsiella pneumonia, Enterobacter aerogenes,* and *Salmonella spp,* albeit found no antibacterial effect against *Shigella dysenteriae*.

A common explanation for the antibacterial activity of *C. aurantifolia* plant extract is due to the lipophilic nature of the phytochemicals present in the plant, which are mostly terpenoids (monoterpenoids and sesquiterpenoids), in which their lipophilic characteristic allows the them and other lipophilic phytochemicals to be dissolved and incorporated into the cell membrane of bacteria and mitochondria, which mostly contains lipid bilayer, and causes cell membrane instability and permeability, further results in the rupture of the bacteria (Al-Aamri *et al.*, 2018; Chi *et al*., 2019; Oikeh *et al.*, 2015). Based on the result, it was also noted that Gram-negative bacteria were considerably more resistant than Gram-positive bacteria, which can be explained by the presence of the outer lipopolysaccharide layer and periplasmic space; outer lipopolysaccharide layer can block the entrance of phytochemicals, while the periplasmic space contains enzymes that could break down some of the phytochemicals (Parama, Sukrama, & Handoko, 2019; Chi *et al*., 2019).

Additionally, other components present may also promote bacterial cell death; phenolics, saponins, and steroids may degrade lipid cell membrane, tannins may inhibit protein synthesis for bacterial peptidoglycan, and flavonoids may inhibit DNA synthesis (Al-Aamri *et al.*, 2018; Chi *et al*., 2019; Oikeh *et al.*, 2015; Parama, Sukrama, & Handoko, 2019). However, these effects may differ depending on the region, species, harvesting condition, and plant part used, which could explain the various results obtained (Chi *et al*., 2019; Oikeh *et al.*, 2015).

Antioxidant

Citrus is known for its antioxidant activity due to its ascorbic acid content, phenolic compounds like flavonoids, tannins, tocopherol, carotenoids and nitrogen compounds including amines, alkaloids, and amino acids, which present at a high level as natural antioxidant components (M *et al.*, 2017, Patil *et al.*, 2009). Nevertheless, some research states that the main compound for antioxidant activities is the phenolic and vitamin C inside the fruit (M *et al.*,

2017). Phenolic exhibits antioxidant action because it has redox properties that may act as reducing agents, hydrogen donors, and metal chelators (Loizzo *et al.*, 2012). The flavonoids, which are the most crucial compound in phenolic, are known to have radical scavenging properties (M *et al.*, 2017). Based on DPPH assay, it showed that the inhibition rate of the lime extract could be in a range of 11.79-56.89%, although it might also depend from the type of the lime that is used (Al Namani *et al.*, 2018). Whereas, based on the FRAP test that was done by Oikeh *et al.* (2015), lime juice shown to possess antioxidant activity, although the level of effectiveness was still below ascorbic acid. Besides the fruit, Citrus leave was also reported to contain some secondary metabolites, such as steroids, tannins, alkaloids, and ascorbic acid, that may also possess antioxidant activities (Al Namani *et al.*, 2018).

Anticancer or antitumor

Cancer, as reported by Global Cancer Statistics, caused 8.8 million deaths in 2015, and WHO estimates 96 million deaths in 2018, and it has become the second-largest cause of death annually, with lung and colorectal are among the highest prevalence cancer types (WHO, 2019; J Esparza-Martínez *et al.*, 2019; Patil *et al.*, 2009; Zhao *et al*., 2017). Common causes of cancer highlight the excessive production of free radicals due to oxidative stress, which surpasses DNA repairment ability and causes mutagenesis and carcinogenesis (J Esparza-Martínez *et al.*, 2019; Şeker Karatoprak *et al.*, 2020). Some common synthetic drugs (i.e. 5-fluorouracil, doxorubicin, cisplatin) had gained resistance or have wide side effects, so natural and less toxic alternative is currently in need, and among these is the *C. aurantifolia* plant (WHO, 2019; J Esparza-Martínez *et al.*, 2019; Şeker Karatoprak *et al.*, 2020; Patil *et al.*, 2009; Zhao *et al*., 2017).

Anticancer or antitumor properties of *C. aurantifolia* have been recently investigated. Zhao *et al*. (2017) reported the usage of the fruit peel that was dried, extracted, and purified for carbohydrate analysis, to showcase the anticancer activity of the carbohydrate phytochemical compound against injected H22 cells in mice; their results reported a very significant decreased in both tumor weight and tumor volume compared to the model group with a dose-dependent relationship, but it also exhibited a slight negative effect in the body weight of mice. Additionally, the carbohydrate extract also significantly increased the amount of tumor-infiltrating lymphocytes (TIL), increased apoptosis signaling due to decline in the G0/G1 phase and G2/M phase in the cell cycle, increased apoptotic genes expression (caspase-3), and decreased anti-apoptotic genes expression (Bcl-xL & Mcl-1); all of these were reported with a dose-dependent relationship.

J Esparza-Martínez *et al.* (2019) utilized methanolic extract of *C. aurantifolia* fruit waste as anticancer agent against colon carcinoma cell (RKO) and HT-29 colon cancer cell lines using MTT assay, and reported a significant decrease in the viability of RKO cell lines with a dose-dependent relationship, while the most optimized result obtained from the freeze-dried fruit waste, with higher temperature and longer exposure time generally enhanced the efficacy Meanwhile, it reported mixed results in the viability of HT-29 cell lines, where 24-hour and 48-hour exposure of 10% phytochemical concentration resulted in a significant decrease of viability, and this effect increased with the drying temperature used; freeze-dried fruit sample also showed significant decreased but

was less effective compared to dried samples. Meanwhile 1% and 5% phytochemical concentration, each obtained from both dried and freeze-dried fruit separately, gave moderate decrease in the same exposure time. All these effects were proposed to be caused by the presence of phenolic compounds such as hesperidin, naringin, and naringenin, which showed a strong and significant positive correlation towards cell line viability (J Esparza-Martínez *et al.*, 2019).

Şeker Karatoprak *et al.* (2020) utilized lyophilized methanolic, ethanolic, and aqueous extract of dried whole fruit for assessing anticancer activity against A549 (human lung cancer cells), MCF7 (human breast cancer cells), and L929 (mouse fibroblast cell line) using MTT assay. The article reported a significant decrease in viability of A549 cell line by 125 - 500 µg/mL of ethanolic extract, 1000 µg/mL methanolic extract, and 2000 µg/mL aqueous extract ($p < 0.01$); a significant decrease in viability of MCF7 cell line by 125 - 500 µg/mL of ethanolic extract, 1000 µg/mL aqueous extract ($p < 0.01$), and 2000 µg/mL methanolic extract ($p < 0.001$); a significant decrease in viability of L929 cell line by 500 - 1000 µg/mL of aqueous extract ($p < 0.05$), 1000 µg/mL methanolic extract and 2000 µg/mL ethanolic extract ($p < 0.01$).

Patil *et al.* (2009) utilized chloroform extract of freeze-dried juice, in which this extract was re-extracted with acetone, methanol, and mixture of methanol: water (8:2) for assessing anticancer activity against pancreatic cancer cells; it reported IC_{50} value of >200 µg/mL for all extract samples after 24-hour incubation, which did not display sufficient effect. Among them, the methanol extract displayed the most potent effect both after 48- and 72-hour incubation based on the lowest IC_{50} value, which was 109.67 ± 4.15 µg/mL when incubated for 48 hour, and 81.20 ± 5.75 µg/mL when incubated for 72 hour. However, it was still far less potent than the pure compounds, which were limonin glycosides, rutin, and hesperidin, which could indicate the involvement of these compounds alone in inhibiting pancreatic cancer cells. Total viable count assay reported a dose-dependent inhibition observed for all extracts, with chloroform extract had the highest potency in inhibiting growth among all after 48-, 72-, and 144-hour incubation, and was considered to be more potent against some pure compounds but was still less potent than tamoxifen acetate, a positive control. Analysis of apoptotic-related protein expression reported cell line given with methanol extract had the highest p53 expression, and highest favor of inducing apoptosis (highest Bax/Bcl-2 ratio), although all extracts also exhibited satisfactory anticancer effect.

Anti-cholesterol

Abnormal cholesterol level is a significant risk factor of cardiovascular diseases, such as coronary heart failure, heart attack, and atherosclerosis, characterized by blockage of blood vessels with fat due to irregularly high total cholesterol (TC), triglyceride (TG), very low-density lipoprotein cholesterol (VLDL-c), and low-density lipoprotein cholesterol (LDL-c) levels, and low high-density lipoprotein cholesterol (HDL-c) levels, and is mainly caused by improper diet and lifestyle (Lin *et al.*, 2019; Boshtam *et al.*, 2013; Cyndi, Andriane, & Nur, 2016). Prolonged consumption of conventional anti-hypercholesterolemic drugs such as statin group can lead to some side effects, so it is generally recommended to incorporate a higher amount of fruits and vegetables into diets, and lime was said to be beneficial for prevention of

this diseases (Lin *et al.*, 2019; Boshtam *et al.*, 2013; Cyndi, Andriane, & Nur, 2016).

Lin *et al.* (2019) used a homogenous mixture of 500 gr powdered peel in 2 L deionized water, which was steam-distilled to retrieve the extract, dried with anhydrous sodium sulfate, and stored in -20^{o}C. The extract was then tested for anticholesterolemic effect with different extract concentrations on mice treated with cholesterol and lard diet; it reported a significant decrease in body weight when treated with a high fat-diet and each of lime essential oil concentration (0.744% and 2.232%) with an observable dose-dependent response. It also reported a significant decrease in serum total cholesterol, serum triglyceride, and LDL-C level, but did not show a significant increase in HDL-C level, nor a significant decrease in liver total cholesterol and liver triglycerides, and only showed a significant increase in fecal sterol content at a higher dosage.

In a study by Boshtam *et al.* (2013), shade-dried and blended lime peel powder and lime juice were used to assess the anti-atherosclerosis effect in rabbits with a high-fat diet, which was divided into four intervention groups, negative control (normal diet), positive control (hypercholesterolemic diet), lime juice-treated + hypercholesterolemic diet, and lime peel-treated + hypercholesterolemic diet groups. The results showed that the groups treated with either lime juice and lime peel extract had a significant decrease in fatty streaks compared to the positive control group, which suggested the success of atherosclerosis inhibition. Meanwhile, Cyndi, Andriane, & Nur (2016) used thick extract retrieved from ethanol-macerated leaf to assess the hypocholesterolemic effect of plant leaf with different dosages towards mice with high cholesterol diet, and reported a significant reduction in the total serum cholesterol for all groups, with the most significant reduction on a dosage of 3.5 g/kg body weight.

High amounts of LDL and VLDL cholesterols are considered to be highly associated with atherosclerosis, in which oxidation of these cholesterols can form lipid peroxides and other reactive oxygen species, which causes more damage to the blood vessel (Lin *et al.*, 2019; Boshtam *et al.*, 2013). High cholesterol content also inhibits HMG-CoA reductase and HMG-CoA synthase, the main enzymes of cholesterol synthesis, and inhibits cellular uptake of cholesterol (Cyndi, Andriane, & Nur, 2016). Lin *et al.* (2019), Boshtam *et al.* (2013), and Cyndi, Andriane, & Nur (2016) suggested that phenolic compounds, particularly flavonoids, present in key lime are the cause of reduction of the LDL and VLDL cholesterols, as flavonoids may act as an antioxidant to reduce reactive oxygen species concentration and may also inhibit cholesterol synthesis and uptake. However, these actions particularly occur due to the complex phytochemical makeup of the plant, which can differ among plant parts and species (Lin *et al.*, 2019; Boshtam *et al.*, 2013; Cyndi, Andriane, & Nur, 2016).

Anti-larvae or anti-mosquito

Lime can be used as anti-larvae, and it is decomposable, free-residue, and eco-friendly. A study by Hamidah & Adrianto (2017) used *Aedes aegypti* larvae to assess anti-larvae activity of *C. aurantifolia*, *C. mitis* and *C. maxima*. Among those three, *C. mitis* showed the highest toxicity level because it could kill 50-95% *A. aegypti* larvae at the lowest concentration, while *C. maxima* had the lowest toxicity level (Hamidah & Adrianto, 2017). The mortality of larvae was evaluated by assessing the damage in the larva's body, either bent

body of the larva, the head cut off, or lengthening of the neck (Adrianto, 2018).

Besides larvicidal, key lime might also be ovicidal. The part of the *C. aurantifolia* that could be used as larvicidal and ovicidal activities were the peel and the leaves. Both the peel and the leaves were extracted into essential oil (Sarma *et al.*, 2019) or using methanol extraction (Adrianto, 2018; Hamidah & Adrianto, 2017; Kumar *et al.*, 2016) for the experiment, while other research used decoction of the leaves (Muniandy, Riswari, & Ruchiatna, 2020). Generally, as the concentration of the extract increased, the mortality of the larva also increased. During the experiment that was done by Muniandy, Riswari, & Ruchiatan (2020), the leaf extract of *C. aurantifolia* resulted in a bigger ovicidal potential than the peel extract in the stage development of the larva. In contrast, the peel extract showed higher larvicidal activity compared to the leaf extract. The secondary metabolite of the key lime methanolic extract was believed to cause toxicity towards the larva, including the alkaloid, flavonoids, steroids, and the terpenoid (Hamidah & Adrianto, 2017). On the other hand, the essential oil of key lime leaves contained citrals that are known to be insecticides, which was toxic for the *A. aegypti* larva (Sarma *et al.*, 2019).

Antidiabetic

Another therapeutic effect that was reported of *C. aurantifolia* is antidiabetic activity (Şeker Karatoprak *et al.*, 2020). In that experiment, the dried fruit was extracted with water (LBW), 70% MeOH (LBM), and EtOH (LBE) in a water bath shaker and lyophilized. The hypoglycemic activity of the plant was studied by *in-vitro* alpha-amylase inhibitory assay at a concentration of 0.5-16 mg/ml. The maximum inhibition was found at concentration of 16 mg/ml. Further analysis revealed that the LBE had high total phenolic content (25.91 GAE/g extract) and flavonoid content (7.83 mg CA/g extract), while the order of the efficiency of the extracts in terms of total flavonoid was LBE > LBW > LBM. Although the total phenolic substance of LBE was more than LBM and LBW, the DPPH radical scavenging activity test showed lower activity. The ABTS radical scavenging methods were conducted at 1 mg/ml, and it was shown that LBW had lower activity than LBE and LBM; with all the extracts showed less activity compared to BHT. Subsequently, the iron reduction activity method showed that the most active extract was LBM. At a concentration of 0.25-2 mg/ml, LBM was seen to be more active than the others, while at a concentration of 0.375 mg/ml, the alpha-amylase inhibition for LBW was found to be 17.83%, 52.07% for LBE, and 67.91% for LBM. It was further shown that LBE had the highest activity against A549 cells, significantly at 1 and 2 mg/ml. In regards of MCF7 cell line, all three extracts significantly inhibited cell viability at 2 mg/ml for LBW (70.65%), LBE (89.56%), and LBM (76.46%). Meanwhile, LBW showed significant inhibition of L929 cell at concentration of 500-2000 µg/ml. Those studies concluded that lime may have an anti-diabetic activity (Şeker Karatoprak *et al.*, 2020).

Anti-inflammatory

Lime is known to be a high source of dietary fibers, phenols, vitamin-C, and flavonoids that were reported to exhibit potential healing properties including anti-inflammatory (Khodabakhsh *et al.*, 2015). A study by Amorim *et al.* (2016) evaluated possible anti-inflammatory effects of the *C. aurantifolia, C. limonia,* and *C. limon* using two methods; the

induced of formalin (2%) paw licking given by subplantar injection in mice, and carrageenan-induced cell migration assay. Based on the first method, the essential oils did not show any anti-inflammatory activity even at higher dosage of 100 mg/kg. However, by using the second method, the anti-inflammatory activity was exhibited by the essential oil of *C. limon* and *C. aurantifolia,* through significant reduction in cell migration after pre-treatment of mice at a dose of 30 and 100 mg/kg. The effects were comparable with dexamethasone as positive control. Further evaluation by measuring the production of Nitric Oxide (NO) and the amount of protein extravasated to the exudate in the cavity showed that *C. aurantifolia, C. limonia,* and *C. limon* essential oils significantly reduced the amount of protein extravasated and NO produced at all doses evaluated similar to the results obtained after pre-treatment of mice with dexamethasone (Amorim *et al.*, 2016).

Anti-cholinesterase

Acetylcholine (ACh) is a neurotransmitter that transfers signal within the central and peripheral nervous systems. Its enzyme, acetylcholinesterase (AChE), hydrolyzes ACh into acetate and choline (Gibb, 2017). Meanwhile, another enzyme known as butyrylcholinesterase (BChE) also able to break down choline-based esters (Jasiecki & Wasag, 2019). The presence of those enzymes could accelerate the neurodegenerative progression of patients with neurological disorders, e.g. Alzheimer's disease (Loizzo *et al.*, 2012). It has been demonstrated that *C. aurantifolia* peel and leaves extract possess potential benefits in anti-cholinesterase activity in a study done by Loizzo *et al.* (2012). Using *in vitro* cholinesterase inhibitory activity assay with AChE and BChE, it was found that methanol and n-hexane extracts of *C. aurantifolia* peel and leaves exhibited a concentration-response relationship, with n-hexane showed the highest activity against AChE (IC_{50} value of 91.4 µg/ml) and BChE (IC_{50} value of 84 µg/ml). It was suggested that the terpenoids present in the extract were the compounds responsible for this inhibitory activity.

ADVERSE EFFECTS

Phytophotodermatitis

Phytophotodermatitis is included as a type of dermatitis, where the inflammation occurs after the skin contact with phototoxic compounds and expose to ultraviolet radiation (Abugroun *et al.*, 2019; Hankinson, Lloyd, & Alweis, 2014). There was a case where a 24-year-old woman had a contact with fresh limes exposed to UV radiation developed the symptoms of phytophotodermatitis in a few hours (Hankinson, Lloyd, & Alweis, 2014). Lime also was reported to contain furocoumarins, a psoralen isomer that may act as a phototoxic compound, and may cause photochemical reaction on the skin that may damage the cell membranes when exposed to the sunlight (Abugroun *et al.*, 2019). Because of the damage, it may result in edema, erythema, blisters, and injury in the epidermis in the form of patches or patterns (handprints or streaks) depending on the plant contact on the skin which can last until a few months.

Toxicity Studies

Thus far, the toxicity studies of key lime had only been done on rabbits. The essential oil of key lime unripe fruits obtained from hydrodistillation was used in a study by Adokoh *et al.* (2019). Essential oils at 50, 100, and 500 mg/kg were tested on rats by oral gavaging for acute single-dose study and sub-chronic

repeated dose toxicity studies. The results showed no acute toxicity nor any effects on the weight. However, in the sub-chronic studies, necrosis, edema, and inflammation on the spleen, liver, and kidneys were observed. There was also an increase in lymphocytes, a decrease in hemoglobin, and high levels of liver enzymes, which showed that the essential oil could cause mild toxicity in the sub-chronic stage. That study suggested that germacrene D, β-pinene, trans-anethole, and linalool identified in the essential oils were responsible for those effects. More study on the toxic effects of the other parts of key lime needs to be done to establish a complete toxicity data of this plant.

CONCLUSION

Studies about the phytochemical screening and pharmacological effects of key lime (*Citrus aurantifolia*) were summarized in this review. Generally, key lime fruit peel, juice, leaves, and stems all contain phenols, flavonoids, steroids, terpenoids, and alkaloids. Key lime exhibited potential pharmacological effects as antibacterial, antioxidant, anticancer, anti-cholesterol, anti-mosquito or anti-larvae, antidiabetic, anti-inflammatory, and anti-cholinesterase. There are potential side effects reported of key lime, such as phytophotodermatitis and possible sub-acute toxic effects. Future clinical studies need to be done to assess and justify the pharmacological effects of key lime in humans.

ACKNOWLEDGMENT

The authors gratefully thank the Indonesia International Institute for Life Science for all the support in paper writing.

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