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Review Article

Chemical Constituents and Biological Activities of *Zanthoxylum limonella* (Rutaceae): A Review

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Abstract

Zanthoxylum limonella belongs to the family of aromatic deciduous trees and shrubs, Rutaceae. In traditional medicine practice, various parts of Z. limonella are used for the treatment of dental caries, febrifugal, sudorific, rheumatism, diuretic, stomach ache and diarrhea. Secondary metabolites have been isolated the stems, stem barks, and fruits. The plant contains alkaloid, amide, lignin, coumarin and terpenoid compounds. The extracts of the various parts, essential oil from the fruits and some pure compounds of Z. limonella have been found to have biological activities, for example, mosquito repellent and mosquito larvicidal, antimicrobial, antioxidant, and antitumour properties. This review compiles some scientific information on botanical description, traditional uses, phytochemical constituents and biological activities of Z. limonella.

Keywords: *Zanthoxylum limonella, Chemical constituents, Biological activities, Antimicrobial, Antioxidant, Antitumour*

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INTRODUCTION

The Rutaceae family comprises almost 150 genera and 1,600 species of trees, shrubs, and climbers distributed throughout the temperate and tropical regions of the world [1]. The chief genera of this family are *Citrus, Zanthoxylum, Ruta, Ptelea, Murraya* and *Fortunella* [2]. Most of the Rutaceae are aromatic plants whose leaves, fruits or cotyledon in seeds contain a complex mixture of volatile aroma compounds [3]. The members of Rutaceae family have been used in perfumery, gastronomy, and traditional medicine. In addition, several publications have reported the presence of secondary chemical constituents of Rutaceae. Phytochemical survey of this family reveals alkaloids, coumarins, flavonoids, limonoids, and volatile oils [4]. Many of these compounds have been associated to different biological activities, for example, antimicrobial [3,5], antidiarrhoeal [6], anticholinesterasic [7], antileishmanial [8], antiprotozoal [9], larvicidal [10,11], and antioxidant activities [12,13]. In family Rutaceae, the genus *Zanthoxylum* has been able to provide a variety of secondary metabolites with interesting phytochemical and biological activities [14].

The genus *Zanthoxylum* comprises over 200 species distributed worldwide in tropical and temperate regions of North America, South America, Africa, Asia, and Australia [15]. The species of this genus are aromatic deciduous trees and shrubs [16]. Members of the *Zathoxylum* have been used in traditional medicines, perfumery and pharmaceutical

industry [14]. Based on the used of these plants in traditional folk medicine reveals a source of valuable bioactive compounds suitable for ethno-pharmaceutical applications. In Asia, *Z. limonella* is commonly known as “ma-kwaen” in Thailand, “kathit-pyu” in Burmese, “chuan hua jiao” in Chinese and “ivy-rue” in India. *Z. limonella* is used in flavouring food and traditional medicine. The different parts of this species are used to treat various diseases, including dental caries, cardiac, respiratory diseases, stomach infections, and rheumatism [17,18]. The present review aims to compile up to date documentations of various scientific papers related phytochemical compositions and biological properties of *Z. limonella*.

BOTANICAL DESCRIPTION

Z. limonella is found in India, Sri Lanka, Thailand, Myanmar, Indo-China, Peninsular Malaysia, Java, the Lesser Sunda Island, Moluccas (Wetar), Sulawesi, the Philippines and southern Papua New Guinea [19]. It is a deciduous, aromatic, medium-sized tree reaching a height of 35 meters (Fig 1a). The green young bark is covered with spines while mature bark is grey with straight or ascending prickles of 2 - 3 cm (Fig 1b). Small prickles occur on the twigs, and all parts of tree have a characteristic lemon-like smell. The leaves are paripinnate or imparipinnate, 30 - 40 cm long. The leaflets are opposite to sub opposite, ovate to elliptical, 7 - 13 cm long, 3 - 5 cm wide, pellucid dots, the margins entire to glandular crenate. Inflorescense panicles have a terminal or axillary, 8 - 14 cm long. The flowers are white or pale yellow, 2 - 3 mm long, 4 sepals and 4 petals. The male flowers have 4 stamina and 1 rudimentary carpel while female flowers with ovary 1 carpellate. The fruit is a follicle, subglobose, 6 - 7 mm in diameter, with 1 seed per carpel, green turning red when ripe. Seeds are hard and black in colour, 5 mm in diameter (Fig 2) [19].

TRADITIONAL USE

The different parts of *Z. limonella* have been used in Thai folk medicine. The bark contains febrifugal, sudorific, and diuretic properties, while the essential oil of fruit is used for treatment of dental caries [20,21]. In India traditional medicine, the bark has been used to treat cardiac, respiratory diseases, tooth infection, stomach infection and rheumatism [17,18]. The fruits are used as spice and the essential oil extracted from the fruits is known as “Mullilam oil” used as anti-inflammatory, antiseptic,

Fig 1: *Zanthoxylum limonella* (a) Appearance of the overall plant and (b) appearance of stem

Fig 2: Fruits of *Zanthoxylum limonella*

anticholera, diarrhoea, hypocholesterolemic, mosquito repellent and soothing agent for dental caries [22,23]. The Kanikkars tribe prepare a paste of hard spines prepared by rubbing them against rock with water and apply the extract to the breast of a nursing mother to relief pain and also to increase milk supply [24]. In the Phillippines, the pounded bark mixed with oil is a good formula to treat stomach ache. In addition, the bark decoction is also taken to treat chest pain and chewed bark applied as antidote for snake bites [19].

PHYTOCHEMICAL CONSTITUENTS

Previous phytochemical investigations on the different parts of *Z. limonella* showed quite wide variety of chemical substances. A number of secondary metabolites, alkaloids, aromatic and aliphatic amides, terpenes, sterols, and phynylpropanoid-lignans and coumarins have been isolated from this plant [25,26]. A variety of compounds found in *Z. limonella* are compiled in Table 1.

The phytochemical analysis of the dichloromethane extract of the stem of *Z. limonella* showed novel quinolone alkaloid, 4-methoxy-3-(3-methyl-2-oxobut-3-enyl)quinolin-2(1H)-one, limonellone (1) including five known compounds such as lignan: (–)-asarinin (2), two aromatic amides: dihydroalatamide (3) and (–)-tembamide (4), a furoquinoline alkaloid: dictamnine (5), and a benzophenanthridine alkaloid: N-nornitidine (6) (Fig 3). Additionally, the structure of these isolated compounds were also identified by ^{1}H, ^{13}C nuclear magnetic resonance (NMR) spectroscopy, chromatography, and melting points with corresponding authentic samples or literature data [27].

Phytochemical studies on the ethyl acetate extract from the stem bark of *Z. limonella* initiated by Somanabandhu *et al* [28] have resulted in the isolation and structural elucidation of main component triterpenoid: lupeol (7) and the minor compounds such as three coumarins: xanthoxyletin (8), osthol (9), scopoletin (10) and one alkaloid: rutaecarpine (11) (Fig 4). Further work done by Charoenying *et al* [29] on the ethyl acetate extract from the fruit of *Z. limonella* have resulted in the isolation of xanthoxyline (2-hydroxy-4,6 dimethoxy acetophenone) (12) as phenolic compound (Fig 5). The structure of this compound was determined by ^{1}H, ^{13}C NMR spectroscopy, and by the comparison of the spectral data with the data reported in the literature.

Z. limonella is an aromatic plant which contains a different terpenic compound [30]. The volatile oils are obtained a complex mixture of terpenic compounds. The fruits are follicle (pericarp) and consist from one to five carpels and each follicle contains one shiny black seed. The aromatic compounds are stored in the pericarps while rich in oil and unsaturated fatty acid accumulate in the seeds [31,32]. Itiipanichpong *et al* [33] used the hydrodistillation to separate volatile constituents and then analyzed them by gas

Table 1: Chemical constituents found in *Z. limonella*

Plant part	Compound	Chemical category	Reference
Stem	Limonellone	Quinolone alkaloid	[27]
	(-)-Asarinin	Lignan	[27]
	Dihydroalatamide	Aromatic amide	[27]
	(-)-Tembamide	Aromatic amide	[27]
	Dictamnine	Furoquinoline alkaloid	[27]
	N-Nornitidine	Benzophenanthridine alkaloid	[27]
Stem bark	Lupeol	Triterpene	[28]
	Xanthoxyletin	Pyronocoumarin	[28]
	Osthol	O-methylated coumarin	[28]
	Scopoletin	Hydroxycoumarin	[28]
	Rutaecapine	Quinazolinocarboline alkaloid	[28]
Fruits	Xanthoxyline	Phenolic compound	[29]
	Limonene	Monoterpene	[33,34,35]
	Terpinen-4-ol	Monoterpene	[33,34,35]
	Sabinene	Monoterpene	[33,34,35]
	3-Carene	Monoterpene	[34]
	α-Terpineol	Monoterpene alcohol	[35]
	β-Pinene	Monoterpene	[35]
	α-Pinene	Monoterpene	[35]
	γ-Terpinene	Monoterpene	[35]
	α-Terpinene	Monoterpene	[35]
	p-Cymene	Alkylbenzene	[35]

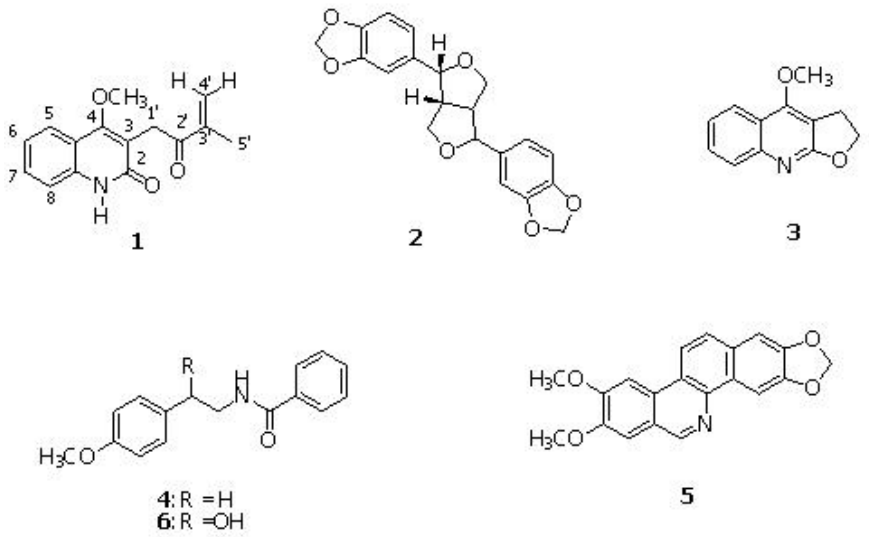

Fig 3: Chemical structure of six compounds (compounds **1-6**) isolated from the stems of *Z. limonella*

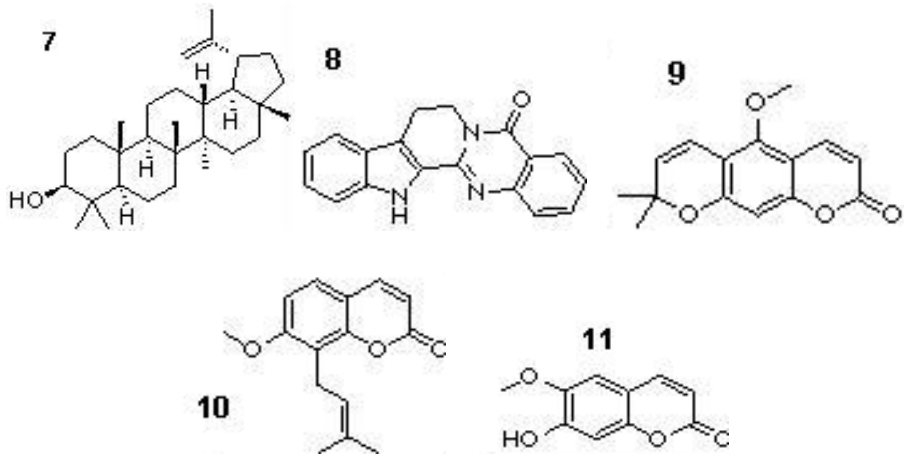

Fig 4: Chemical structure of five compounds (compounds **7-11**) isolated from the stem bark of *Z. limonella*

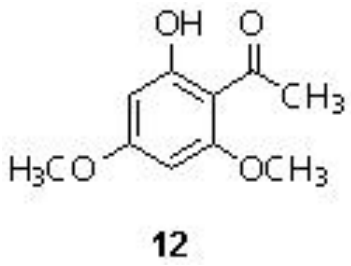

Fig 5: Chemical structure of xanthoxyline (compound **12**) isolated from the fruits of *Z. limonella*

chromatograph coupled to a mass spectrometer detector (GC-MS). Thirty-three chemical components were found in the essential oil distilled from the fruit of *Z. limonella* in Thailand. Limonene (13) (31.09 %), terpinen-4-ol (14) (13.94 %) and sabinene (15) (9.13 %) were found to be the major components (Fig 6). Tangjitjaroenkun *et al* [34] identified 22 compounds from fruit volatile oil by GC-MS, representing 97.3 % of the total components. A 12 % oil yield was obtained on a dry weight basis. The fruit volatile oil is made up largely of monoterpene hydrocarbons (88.34 %) and oxygenated monoterpenoids (8.26 %) and sesquiterpenoids (1.4 %). The major constituents were sabinene (42.73 %), limonene (39.05 %), and terpinen-4-ol (5.40 %), 3-carene (16) (2.70 %) (Fig 6) [34]. In another study, Jirovetz *et al* [35] collected *Z. limonella* seeds from Kerala, southern India, and extracted them by steam distillation method. Forty-one different compounds could be identified in the essential oil of seed representing 98.3 % of the oil composition. The seed oil was rich in sabinene (47.12 %), alpha-terpineol (17) (7.73 %), terpinen-4-ol (6.61 %), beta-pinene (18) (5.99 %), limonene (4.06 %), alpha-pinene (19) (3.87 %), gamma-terpinene (20) (3.64 %), alpha-

terpinene (21) (3.45 %) and para-cymene (22) (3.08 %) (Fig 6). Concerning the comparison of the majority volatile oils constituents from fruits collected in three different localities reveal monocyclic monoterpenes such as sabinene (9.1 %, 42.73 %, 47.12 %), limonene (31.1 %, 39.05 %, 4.06 %) and terpinen-4-ol (13.9 %, 5.40 %, 6.61 %). Thus, variation of environmental, ecological, geographical conditions as well as culture conditions and extraction techniques results in a diversity of volatile oil chemical components. Such conditions are likely to affect the biosynthetic pathway of the plant resulting in diverse essential oils.

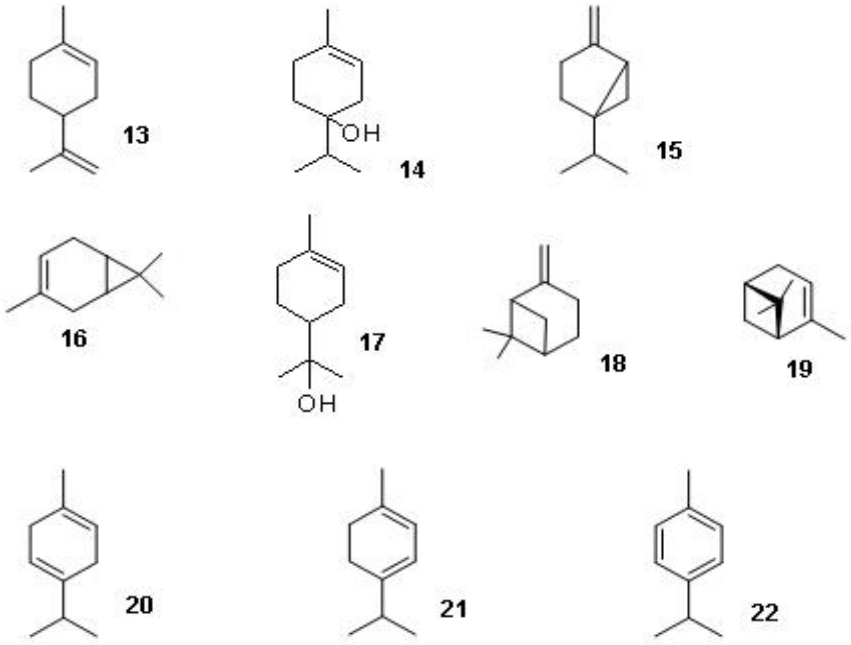

Fig 6: Chemical structure of volatile compounds (compounds 13-22) of *Z. limonella* fruits essential oil

BIOLOGICAL ACTIVITIES

The biological and pharmacological activities, mosquito repellent and larvicidal activity, antimicrobial activity, antitumour activity, have been frequently reported and detailed in Table 2.

Mosquito repellent and larvicidal activity

Essential oil and petroleum ether extract of the fruits of *Z. limonella* have been evaluated as repellent against *Aedes albopictus* mosquito in both a mustard and coconut oil. All repellents have been tested at three different concentrations (10, 20 and 30 %). The fruit oil and extract in mustard oil provide better protection than in coconut oil. The fruit oil offered the longest duration of protection effective against mosquitoes. Repellent efficacy of 30 % concentration of fruit oil in mustard oil mixture gave the longest protection time of repellency (296 - 304 min) against *A. albopictus* [23]. In another study, Trongtokit *et al* [21] investigated the repellency of essential oil from the fruit of *Z. limonella* using an arm-in-cage test. The undiluted fruit volatile oil gave complete protection time for 2 h against *A. aegypti, Culex quinquefasciatus*, and *Anopheles dirus*. Moreover, Mostel, the repellent product by the Insecticide Research Unit, Mahidol University, Thailand, containing binary mixture of 10 % clove oil (essential oil from *Syzygium aromaticum*) plus 10 % makaen oil (essential oil from the fruit of *Z. limonella*) in a gel form gave 100 % protection for 4-5 h against *A. aegypti, C. quinquefasciatus*, and *A. dirus* by arm in cage methods [36]. Mostel provides protection against *A. stephensi* for 4.5-5 h by the cage test and gave protection from free flying mosquitoes in a mosquito proof room for 7-8 h [21].

The essential oil from the fruit of *Z. limonella* was effective against fourth instar larvae of *A. aegypti* in 24 h with the lethal concentration of 50 % and 95 % mortality (LC_{50} and LC_{95}) values were 24.61 and 55.81 ppm, respectively [37]. Rabha *et al* [38] also found that the essential oil hydrolate of *Z. limonella* exhibited strong activities against *A. albopictus* and *C. quinquefasciatus* larvae after 24 h. The LC_{50} and LC_{90} values of *Z. limonella* oil were 11 and 19.4 (% v/v), respectively against *A. albopictus* larvae and

15.5 and 25.8 (% v/v) against *C. quinquefasciatus* larvae.

Antimicrobial activity

Z. limonella has been used medicinally for the treatment of infectious diseases. It has been reported to contain antibacterial and antifungal activities, which has been investigated *in vitro*. The crude chloroform extract from the fruits of *Z. limonella* showed antituberculous activity against *Mycobacterium tuberculosis* H37Rv with minimum inhibitory concentration (MIC) of 200 µg/mL [39]. Wannissorn *et al* [40] has evaluated antibacterial activity of essential oil from the fruits of *Z. limonella* against 5 enteropathogens, *Salmonella typhimurium* TISTR 292, *S. enteritidis* DMST 17368, *Escherichia coli* TISTR 292, *Clostridium perfringens* DMST 15191 and *Campylobacter jejuni* DMST 15190. The results from disk diffusion assay indicated that the essential oil had antibacterial activity against all the tested bacterial strains. Maximum activity was observed against *C. perfringens* (27.0 mm), followed by *S. typhimurium* (20.5 mm), *C. jejuni* (18 mm), *S. enteritidis* (16.3 mm) and *E. coli* exhibited weak inhibitory activity (13.5 mm).

The bioactive compounds isolated from dichloromethane extract of stem of *Z. limonella* are dictamnine and tembamide [27]. Dictamnine (5) has shown antitubercular activity against *Mycobacterium tuberculosis* H37Rv with a MIC value of 30 µg/mL [41] and shows antibacterial activity against oral pathogens, *Streptococcus sanguinis, Streptococcus mutans*, and *Lactobacillus casei* with MIC of 0.4, 0.4 and 0.1 mg/mL, respectively [42]. It has also been shown to have antifungal activity against the plant pathogenic fungus *Cladosporium cucumerinum* and *Pyricularia oryzae* with MIC of 25 and 6.25 µg/mL, respectively [43,44]. Tembamide (4) exhibits anti-HIV property with EC_{50} values of < 0.1 µg/mL [45].

Nanasombat and Wimuttigosol [46] have investigated the antibacterial and antifungal activities of essential oil obtained from the fruits of this plant against 6 bacterial species (*Bacillus cereus* DMST 5040, *Escherichia coli* DMST 4212, *Listeria monocytogenes* DMST 11256, *Salmonella* Rissen DMST 7097, *Pseudomonas fluorescens* TISTR 358 and *Staphylococcus aureus* TISTR 118), 6 species of yeasts (*Candida lipolytica* TISTR 5655, *Hanseniaspora uvarum* TISTR 515, *Pichia membranaefaciens* TISTR 5093, *Rhodotorula glutinis* TISTR 5159, *Schizosaccharomyces pombe* TISTR 509 and *Zygosaccharomyces rouxii* TISTR 5044) and 4 species of fungi (*Aspergillus flavus* TISTR 3041, *Aspergillus ochraceus* TISTR 3557, *Aspergillus parasiticus* TISTR 3276 and *Fusarium moniliforme* TISTR 3175) and also evaluated by disc diffusion assay and the MIC determination.

Their results showed that the fruits essential oil had inhibitory activity against all of tested strains. This oil was found to be more effective to tested yeasts and fungi as compared to the bacterial strains. The essential oil possessed strong inhibitory activity against tested yeasts such as *Rhodotorula glutinis, Schizosaccharomyces pombe, Hanseniaspora uvarum* with the large zone of inhibition (42.3, 34.0 and 33.3 mm) and the MIC values of 1, 2 and 1 mg/mL, respectively. In this study, the essential oil had inhibitory activity against all tested fungi, especially *Aspergillus ochraceus* and *Fusarium moniliforme* with inhibition zones of 25.8 and 23.5 mm and the MIC values of these tested strains were equivalent at 1 mg/mL. The antibacterial activity of essential oil was also observed on the bacteria *Bacillus cereus* and *Staphylococcus aureus* with the wide zone of inhibition (20.5 and 21.5 mm) and the MIC values for these bacteria were the same (6 mg/mL).

In another study, Tangjitjaroenkun *et al* [24] reported that the antibacterial activities of crude essential oil, the distilled fractions (fraction I, fraction II, and fraction III) from *Z. limonella* fruits and pure major compounds (sabinene, limonene and terpinen-4-ol) were tested against various bacteria (*B. subtilis* ATCC 6633, *S. aureus* (ATCC 25923; methicillin –sensitive *S. aureus* (MSSA)), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853)) by paper disk diffusion method with a final concentration of each test sample of 5 mg per disk. The crude oil, fraction I, fraction II and sabinene showed a potent antibacterial activity against *B. subtilis, S. aureus* and *E. coli* (> 10 mm inhibition diameter) but weak against *P. aeruginosa* (< 10 mm inhibition diameter). The crude oil exhibited higher antibacterial activity than distilled fractions and sabinene, suggesting that the untested minor components in the crude oil might have synergistic or additive effects against the tested bacteria. In addition, the crude oil and major compounds, sabinene, were further examined the antibacterial activity against the drug-sensitive strains (MSSA, *E.coli, P. aeruginosa*) and multi-drug resistant (MDR) bacteria of *S. aureus* ATCC 43300 (methicillin-resistant *S. aureus* (MRSA)), extend spectrum β-lactamase (ESBL)-producing *E. coli* and the clinical isolated MDR *P. aeruginosa* using the quantitative microdilution susceptibility assay. The essential

Table 2: Reported biological activities of *Z. limonella*

Preparation, Compound	Plant part	Effect	Model	Value of detection	Reference
Mosquito repellent and larvicidal activity					
essential oil	fruits	duration of protection against *Aedes albopictus*	arm in cage test	296-304 minutes	[23]
essential oil	fruits	duration of protection against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus*	arm in cage test	120 minutes	[21]
essential oil	fruits	lethal concentration against *A. aegypti*	mosquito rearing technique	LC_{50} = 24.61ppm LC_{95} = 55.81 ppm	[37]
essential oil	fruits	lethal concentration against *A. Albopictus* and *C. quinquefasciatus*	mosquito rearing technique	LC_{50} = 11.0 and 15.5 %(v/v) LC_{95} = 19.4 and 25.8 %(v/v)	[38]
Antibacterial activity					
crude chloroform extract	fruits	growth inhibition against *Mycobacterium tuberculosis* H37 Rv	serial dilution method	MIC = 200 µg/mL	[39]
essential oil	fruits	zone inhibition against *Clostridium perfringens* DMST 15191, *Salmonella typhimulium* TISTR 292, *Campylobacter jejuni* DMST 15190, *S. enteritidis* DMST 17368, *Escherichia coli* TISTR292,	disc diffusion method	27.0, 20.5, 18, 16.3 and 13.5 mm (at 15 µL/disc)	[40]
essential oil	fruits	zone inhibition against *Bacillus cereus* and *Staphylococcus aureus*	disc diffusion method	20.5 and 21.5 mm (at 10 µL/disc)	[46]
essential oil	fruits	growth inhibition against meticillin-sensitive *Staphylococcus aureus*, *E. coli* ATCC 25922, meticillin-resistant *S. aureus* and extend spectrum β-lactamase-producing *E. coli*	serial dilution method	MIC = 0.25, 0.5, 0.5 and 1.0 g/L MBC = 2.0, 2.0, 2.0 and 2.0 g/L	[24]
sabinene (major compound in essential oil)	fruits	growth inhibition against meticillin-sensitive *Staphylococcus aureus*, *E. coli* ATCC 25922, meticillin-resistant *S. aureus* and extend spectrum β-lactamase-producing *E. coli*	serial dilution method	MIC = 4.22, 4.22, 4.22 and 4.22 g/L MBC = 16.88, 16.88, 16.88 and 16.88 g/L	[24]
dictamnine (**5**)	stems	growth inhibition against *Mycobacterium tuberculosis* H37 Rv	serial dilution method	MIC = 30 µg/mL	[41]
dictamnine (**5**)	stems	growth inhibition against *Streptococcus sanguinis, S. mutans* and *Lactobacillus casei*	serial dilution method	MIC = 0.4, 0.4 and 0.1 mg/mL	[42]

Table 2: *Reported biological activities of Z. limonella (continued*

Preparation, Compound	Plant part	Effect	Model	Value of detection	Reference
Antifungal activity					
essential oil	fruits	growth inhibition and zone inhibition against *Rhodotorula glutinis*, *Schizosaccharomyces pombe*, *Hanseniaspora uvarum*, *Aspergillus ochraceus* and *Fusarium moniliforme*	disc diffusion method and serial dilution method	42.3, 34.0, 33.3, 25.8 and 23.5 mm (at 10 μL /disc) MIC = 1, 2, 1, 1 and 1 mg/mL	[46]
dictamnine (**5**)	stems	growth inhibition against *Cladosporim cucumerinum* and *Pyricularia oryzae*	serial dilution method	MIC = 25 and 6.25 μg/mL	[43][44]
Antiviral activity					
tembamide (**4**)	stems	inhibition of HIV replication in H9 lymphocyte cells	Anti HIV assay	EC_{50} = <0.1 μg/mL	[45]
Anitoxidant activity					
essentail oil	fruits	diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and trolox equivalent antioxidant capacity (TEAC)	DPPH radical scavenging activity assay and TEAC assay	IC_{50} = 5,764.67 ± 6.45 μg/mL TEAC = 7.05 ± 0.34 μM	[53]
essential oil	fruits	DPPH radical scavenging, β-carotene bleaching, reduction of ferric to ferrous ions, superoxide anion scavenging	(1) DPPH radical scavenging activity assay, (2) *β*-Carotene bleaching test, (3) Ferric reducing antioxidant power (FRAP) assay and (4) Superoxide anion scavenging activity assay	(1) IC_{50} = 5.66 mg/mL (2) antioxidant acitivity = 66.16% (3) concentration of ferrous tripyridyltriazine = 0.26 mM/mg (4) inhibition of superoxide anion = 79.07%	[46]
crude dichloromethane extract	stems	DPPH radical scavenging and trolox equivalent antioxidant capacity (TEAC)	DPPH radical scavenging activity assay and TEAC assay	IC_{50} = 54.63 ± 2.89 μg/mL TEAC = 15.47 ± 0.34 μM	[53]
crude methanol extract	stems	DPPH radical scavenging and trolox equivalent antioxidant capacity (TEAC)	DPPH radical scavenging activity assay and TEAC assay	IC_{50} = 117.47 ± 4.66 μg/mL TEAC = 14.34 ± 0.31 μM	[53]
aqueous extract	seeds	trolox equivalent antioxidant capacity (TEAC)	TEAC assay	TEAC = 5.05 mM/gdw	[54]
Antitumour activity					
crude methanol extract	fruits	inhibition of Epstein-Barr virus (EBV) activation in Raji cells	Epstein-Barr virus early antigen (EBV-EA) activation assay	Inhibitory effect (IE) = ≥ 70% (at 200 μg/mL)	[55]

oil, the result showed that the two sensitive strains (MSSA and *E. coli*) had the MIC values (0.25 and 0.5 g/L, respectively) and the resistant strains (MRSA, ESBL) had the MIC values of 0.5 and 1.0 g/L, respectively. The same minimum bactericidal concentration (MBC) values of 2.0 g/L showing the crude oil to contain the bactericidal effect to these tested strains. In addition, the MIC and MBC values of pure sabinene against MSSA, *E.coli*, MRSA, and ESBL were equivalent at 4.22 and 16.88 g/L, respectively. In contrast, crude oil and sabinene displayed the undectectable MIC and MBC values of drug-sensitive and MDR strains of *P. aeruginosa* at the highest concentration tested. In this study, the bactericidal activities of essential oil and sabinene were determined by using an *in vitro* time killing assay at dose equivalent to MBC, 2 × MBC and 4 × MBC, respectively. The trend of time-kill curves of crude oil and sabinene appears to be both time- and concentration-dependent. At higher concentration (2 × MBC and 4 × MBC) and longer contact time of interaction, more bacteria were killed. The essential oil and sabinene at the concentration of 4 × MBC (8 and 67.6 g/L, respectively) killed MSSA, *E. coli*, ESBL-producing *E. coli* and MRSA within 3, 6, 15 and 60 min, respectively. The effect of crude oil and sabinene were rapidly bactericidal at 2 × MBC (4 g/L and 33.8 g/L) achieving a complete elimination of MSSA and *E.coli* within 10 min and MRSA and ESBL-producing *E.coli* within 90 min exposure time. Considering that the essential oil had a higher bactericidal acitivity than sabinene, and the MBC values of the sabinene were eight times higher than those of crude oil. These results indicated that the essential oil is a complex mixture of wide variety compounds and their active components might have a synergistic or additive effect against tested organisms.

Antioxidant activity

Oxidants/free radicals relevant to human physiology are mainly derived from oxygen (reactive oxygen species/ ROS) and nitrogen (reactive nitrogen species/ RNS). They are produced intracellulary in the body, by endogenous sources, and also from the environment, man-made sources, and exogenous sources [47,48]. Free radicals can damage the cellular components such as DNA, cell membrane lipids and modify several biochemical compounds [49]. Free radicals has been linked to the various diseases including cancer, cardiovascular disease, stroke, atherosclerosis, arthritis, Alzheimer's disease, Parkinson's disease, diabetes and the ageing process [50]. Antioxidants are substances that neutralize free radicals and protect cell constituents from oxidative damage caused by unstable molecules. These antioxidants may be synthesized in the body or obtained from plants such as fruits, vegetable, spices and herbs [51]. Plants produce a wide variety of substances that possess antioxidant activity [52].

Tangjitjareonkun *et al* [53] reported that free radical scavenging activity of *Z. limonella* essential oil from the fruits and crude dichloromethane extracts of leaves (LD) and stems (SD); as well as methanol extracts of leaves (LM) and stems (SM) in both cell free and cell-based systems. The quantitative screening of antioxidant activity of extract and essential oil were evaluated by determining its effect on diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and trolox equivalent antioxidant capacity (TEAC). In DPPH antioxidant assay, IC_{50} values of SD, SM and essential oil were 54.63 ± 2.89, 117.47 ± 4.66 and 5,764.67 ± 6.45 µg/mL, respectively, which was comparable to the standard antioxidant butylated hydroxyltoluene (BHT) (IC_{50} value = 19.70 ± 0.2 µg/mL). TEAC values of SM, SD and essential oil were 15.47 ± 0.34, 14.34 ± 0.31 and 7.05 ± 0.34 µM, respectively. These data showed the ranking order of free radical scavenging activity, SM > SD > essential oil. However, the LD and LM were not determined the free radical scavenging activity due to the low antioxidant activity. Furthermore, the antioxidant capacity of *Z. limonella* crude extracts (SD, SM) and essential oil were investigated on prostate adenomacarcinoma cell lines, PC-3 and DU-145. All extract (SD, SM) and essential oil at 0.5, 5 and 10 µg/mL exhibited weak activity to reduce the malondialdehyde (MDA) level in both normal (untreated) cell lysates [53]. These results indicated that the extracts and essential oil cannot provide direct protection against free radical to protect the cell membrane from the damage caused by lipid peroxidation. On the other hand, the pretreated PC-3 and DU-145 with various concentrations of SD, SM, and essential oil at 0, 0.5 and 5 µg/mL for 24 h significantly decreased the MDA level of cell lysate. The intracellular antioxidant system, glutathione (GSH) and catalase (CAT) levels of cell lysates from PC-3 and DU-145 pretreated with SD and SM seemed to be significantly increased when compared to the control group, while the pretreated PC-3 and DU-145 with essential oil at all concentrations expressed high CAT level in dose-dependent manner more than GSH level in both cell lines. The SD and SM might have effect on levels of GSH and CAT, while essential oil may play a role in the regulation of CAT activity. These data suggested that the crude extracts,

SM and SD, and essential oil may also regulated by increasing expression of the gene encoding the endogenous antioxidants, GSH and CAT.

In another study by Nanasombat and Wimuttigosol [46] the essential oil from the fruits of *Z. limonella* was determined by four different methods and exhibited strong antioxidant activity with IC_{50} value of 5.66 mg/mL (DPPH assay), 66.16 % antioxidant activity (β-carotene bleaching test), 0.26 mM/mg reducing capacity (ferric reducing antioxidant power assay), and 79.07 % (superoxide anion scavenging activity assay). In addition, the free radical scavenging activity of the aqueous seed extract of this plant was measured by TEAC assay and the TEAC value was 5.059 mM trolox/gdw [54].

Antitumour activity

In vitro assessment of the antitumour promoting activity of methanolic fruit extract of *Z. limonella* was measured by the *in vitro* 12-O-tetradecanoylphorbol-13-acetate (HPA, 40 ng/mL) induced Epstein-Barr virus early antigen (EBV-EA) activation assay in lymphoid cell line, Raji cells. The fruit extract showed strongly inhibitory effect (IE ≥ 70 %) at 200 µg/mL. The result indicated that this extract might contain effective antitumour promoters or cancer chemopreventive agents [55].

CONCLUSION

Z. limonella is used as folk medicine and flavouring food. The present review describes the botanical description, traditional uses, phytochemical constituents and biological activities. Available scientific literature shows that diverse secondary metabolites have been isolated from bark, stem, root and fruit of *Z. limonella* such as alkaloids, amides, terpenoids, coumarins, sterols, volatile oil and others. Various reports indicate that many of these phytoconstituents exhibit biological activities such as mosquito repellent and larvicidal, antimicrobial, antioxidant, and antitumour properties. Thus, there is little doubt about the potential of *Z. limonella* as an important resource for novel therapeutic agents. Therefore, this literature review may provide assistance to researchers who wish to further investigate the pharmacological properties of the plant.

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