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A COMPREHENSIVE REVIEW ON PHYTOCHEMICALS AND PHARMACOLOGICAL ACTIVITY OF *LIMONIA ACIDISSIMA* L. FRUIT

A. R. Sowjanya, Vageesh Revadigar, T. Tamizh Mani *, L. Shiju and T. Pavithra

Department of Pharmacognosy, Bharathi College of Pharmacy, Bharathinagara, Mandya - 571422, Karnataka, India.

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Correspondence to Author:

T. Tamizh Mani

Department of Pharmacognosy,
Bharathi College of Pharmacy,
Bharathinagara, Mandya - 571422,
Karnataka, India.

E-mail: sowjanyaar1998gowda@gmail.com

ABSTRACT: The tropical fruit plant *Limonia acidissima* L. commonly known as wood apple which belongs to the *Rutaceae* family, is well known for its therapeutic qualities. The edible fruits and other components of the wood apple, *Limonia acidissima* Linn., are used in a variety of culinary preparations. Different plant parts are used to cure different illnesses in traditional medicine. The plant was studied for its potential as a medicine in addition to being used as a folk remedies in India. The aqueous, methanolic, and ethanolic extracts of fruit pulp have diverse secondary metabolites like tannin, saponin, flavonoids, phenolic compounds, reducing sugars, alkaloids, etc. Numerous scientific research have confirmed the ethnomedical effects of several bioactive substances with antidiabetic, antihyperglycemic, antibacterial, anticancer, hepatoprotective, and other activities. The current review focuses on Pharmacological and Ethnobotanical uses of *Limonia acidissima* L. to provide updated information of this fruit.

INTRODUCTION: The tropical fruit plant *Limonia acidissima* L., which belongs to the *Rutaceae* family, is well known for its therapeutic qualities. It is one of the sacred tree that is placed in gardens and temples. This plant is used as medication to cure a number of illnesses. The plant's roots, fruits, bark, and leaves are its valuable portions and are used for a variety of medicinal uses. People can make syrups, beverages, jellies, and jams out of its sticky pulp, since it is edible ¹. It is an upright, slow-growing, deciduous tree with a few upward-extending branches that bend outward

close to the summit, where they split into thin branchlets that droop at the tips. The leaves are leathery, dark green, alternating, and deciduous. On some of the zigzag twigs, the spines are axillary, short, and straight whereas tiny, many, dull-red or greenish flowers are born in terminal or lateral panicles that are loose in nature. The berry has a tough, woody rind that is roughly 6 mm thick and has a greyish-white, scurfy texture. The pulp has white seeds strewn throughout and is sticky, dark, odorous, resinous, astringent, and acidic or sweet ².

Flavonoids, Glycosides, Saponins, and Tannins are present in wood apple fruit. Some Coumarins and Tyramine derivatives have reportedly also been extracted from *Limonia* fruits. In comparison to many other fruits, it is a nutrient-dense fruit with a surprisingly high protein content and low levels of sugar and carbohydrates. Beta carotene, a precursor



of vitamin A, is abundant in *L. acidissima* L., which also has high levels of the Vitamins B such as Thiamine, Riboflavin, in inclusive of fruit also contains Vitamin C. Carotenoids and Flavonoids are few among the many phytochemicals that possess significant activity.

These class of phytochemicals significantly reduce oxidative damage at cellular level hence capable of providing relief in various chronic diseases. Thus they are important³.

TABLE 1: BOTANICAL DISTRIBUTION OF WOOD APPLE^{2, 6}

Family	<i>Rutaceae</i>
Kingdom	Plantae
Sub-Kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Genus	<i>Limonia</i> L.
Species	<i>L. acidissima</i>

Synonyms: *Feronia elephantum* Correa, *Schinus limonia* L. *Feronia limonia* (L.) Swingle.

Fruits Macroscopy:

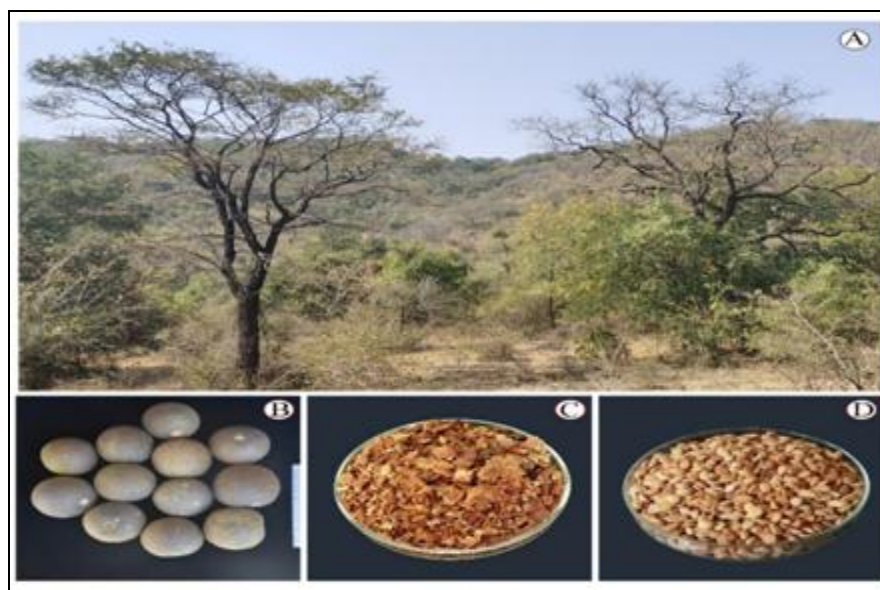


FIG. 1: WOOD APPLE (*LIMONIA ACIDISSIMA* L.)

(A) Tree habit

(B) Fruits

(C) Pulp

(D) Seeds

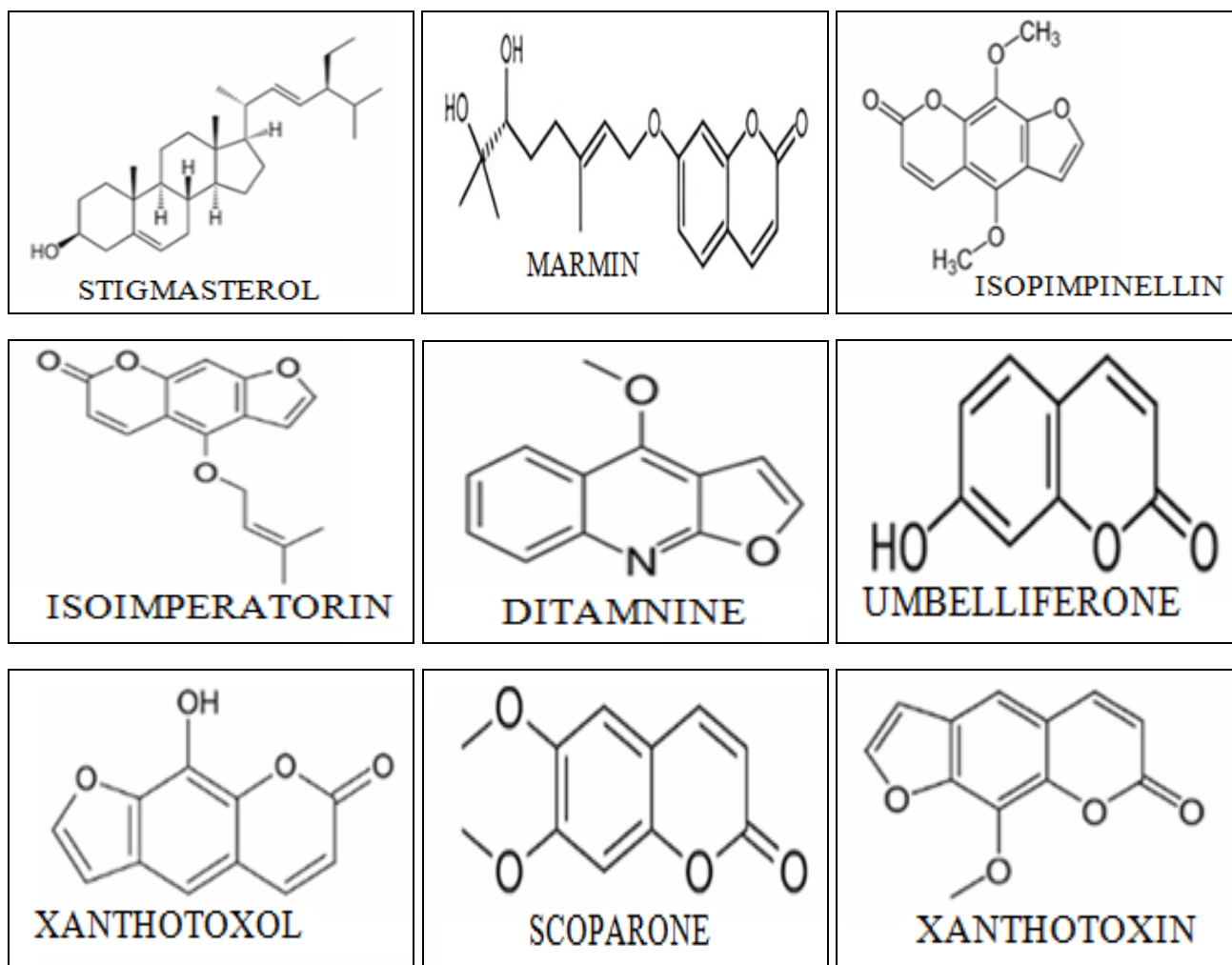
TABLE 2: VERNACULAR NAMES FOR WOOD APPLE¹²

Languages	Names
Hindi	Kabeet, Kath Bel, Kaitha
English	Wood apple, Monkey fruit, Elephant apple, Curd fruit
Marathi	Kauth, Kavath, Kaveet, Kovit, Sit-ranlimbi
Sanskrit	Kapittha or Dadhistha
Telugu	Vellega pandu, Kapithhamu, Pulivelaga, Parupuvelaga
Gujarati	Kothu
Bengali	Koth bel
Malyalam	Cerukantunarakam, Cherrukatnarragam, Vilam kai
Tamil	Vilanga, Narivila, Vilaa, Nilavila, Vilam Palam
Urdu	Kaitha
Tibetian	Kapita
Kannada	Aruna mullu
Oriya	Kaitha

Chemical Constituents Reported from Different Parts of *Limonia acidissima* L. Fruit: Polyphenols, Vitamins, Saponins, Coumarins, Amino acids, Tri-terpenoids, Phytosterols, Tannins, Flavonoids, Steroids, Glycosides, Fat, Calcium, Magnesium, Iron, Umbelliferone, Dictamnine, Xanthotoxol, Scoparone, Xanthotoxin, Isopimpinellin, Isoimperatorin and Marmin⁴.

and is dark, fragrant, resinous, acidic, or sweet⁵. Stigmasterol is found in unripe fruits. Citric acid, along with other fruit acids, mucilage, and minerals, are abundant in fruit pulp. The pericarp has been shown to contain sterols, fatty acids,

coumarins, and alkaloids. Additionally, it contains Marmin, Isopimpinellin, Isoimperatorin, Ditamnine, Umbelliferone, Xanthotoxol, Scoparone, and Xanthotoxin⁶.



Three distinct stages of wood apple fruits, unripe, semiripe, and ripewere examined for biochemical alterations. Similar to the majority of ripening fruits, the main alterations were a rise in total sugars and a fall in acidity. High performance liquid chromatography (HPLC) studies have shown that Citric acid is the main organic acid, which is found in the highest concentrations in unripe fruits and the lowest in ripe ones. In contrast to other fruits, the soluble solids decreased as the fruit ripened. However, ripe fruit consists higher levels of total protein and phenolics. There is a strong association between the Phenolics concentration and the freeradicle reducing power, the antioxidant activity of wood apple fruit pulp is due to phenolics which has capacity to neutralise free radicles⁷.

Ethno-Medical Uses: both the traditional and folk systems of indigenous medicine, *Limonia acidissima L.* is found to have therapeutic properties. Tribal people have utilized every component of the plant for a variety of purposes such as to lessen gum and throat infections. Ripe fruit pulp is consumed with sugar to get cure of sore throats. Fruit pulp aids in the treatment of bleeding gums and reduces foul breath. It is also used to treat sterility brought on by a lack of progesterone hormones. Regular morning use of pulp acts as medicine to tone up the sagging breast and uterine. The Ayurvedic medical system uses the unripe fruits to sour, fragrant, astringent in taste and alexipharmic which are cure pharyngodynia, pruritus, and diarrhea. Seeds are used to treat

cardiac conditions, and unripe fruits are astringent. Seeds are used to cure dysentery and diarrhea⁸. Its biological actions are diverse which include hepatoprotection, adaptogenic activity, and treatment of jaundice, dyspepsia, leucorrhoea, and blood impurities. Fruit is used in India as a liver and heart tonic, to treat diarrhea and dysentery, and as a successful remedy for gum disease, hiccups, and sore throats⁹.

Herbal remedies in some parts of world make use of the leaves and fruits to treat a number of bone and joint conditions, bilious disorders, capillary bleeding prevention, colds, influenza, piles, dysentery, chronic constipation, and scurvy¹⁰. Fruit pulp combined with cardamom, honey, and cumin seeds is used as a medication to treat conditions like piles, indigestion, diarrhea, and malnutrition-induced liver cirrhosis in children. It has been reported that rubbing ripe pulp can reduce poisonous string pain¹¹.

Medicinal Uses: The fruit contains fruit acids, vitamins and minerals. It is used mainly as a liver tonic to stimulate the digestive system. The fruit is also astringent, especially when unripe, and a cardiac tonic. The pulp of the fruit, especially when unripe, is used in the treatment of diarrhoea and dysentery. The fruit is also reported as an effective to having the treatment of the hiccough, sore throat and diseases of the gums. Both the fruit pulp and the powdered rind can be poulticed onto the bites and stings of venomous insects. The fruit is part of a formula that is applied as a paste to the breasts in order to tone them¹².

Nutritional Composition and Phytochemicals: In wood apple extracts contain variety of active constituents like phenols, flavonoids, saponins, terpenoids, tannins, fats steroids, gums mucilages, fixed oils, alkaloids, glycosides which are reported that are responsible for various medicinal activities of wood apple fruit. Different parts of the *Limonia acidissima* plant like its leaves, roots, bark and fruits are used in the treatment of variety of ailments like dysentery and diarrhea from ancient times. As fruits supply minerals, vitamins, proteins and carbohydrates they are considered as protective foods. Ripened fruit pulp contains seeds which are embedded in pulp which is about 70% of total weight. Fruit pulp contains about 1.9% mineral

matter, 70% moisture, 2.3% acidity, 7.3% protein, 0.6% fat, 0.07% iron, 7.2% sugars, 0.08% phosphorus, 7.2% sugars, and it is valuable source of calcium (0.17%) and riboflavin (77 mg / 100 g). The fruits also contain phytochemicals like saponins, polyphenols, tannins, amino acids, coumarins, phytosterols vitamins, amino acids, terpenoids etc. Pectin content present in fruit is 3 to 8%¹³.

TABLE 3: NUTRITIONAL ANALYSIS OF DRIED PULP

Constituents	Dried pulp (%)
Dietary fiber	1.7
Fat	4.3
Ash	5.28
Moisture	6.4
Protein	13.8
Carbohydrates	70.0

Source: Pandey et al. (2014).

TABLE 4: MINERALS AND VITAMINS CONTENT OF *L. ACIDISSIMA* PULP

Analyte	Concentration(µg/ g)
Minerals(µg/ g)	
P	1,137.53
Mg	852.5
Ca	711.8
Fe	23
Zn	23.84
Cu	6.67
Mn	3.64
Sb	0.626
As	ND
Be	ND
Cd	ND
Cr	1.543
Co	ND
Pb	0.163
Li	0.241
Mo	0.263
Ni	0.819
Se	0.768
Sr	ND
TI	1.930
Ti	0.257
Sn	0.474
Vitamins (µg/ g)	
Vitamin C	180
Riboflavin (B2)	0.23
Thiamine (B1)	0.31
Beta-carotene	0.04

Source: Pandey et al. (2014).

Pharmacological Activity

Antidiabetic Activity: Hydroalcoholic fruit pulp extract of *L. acidissima* (100, 200, and 400 mg/kg) administered orally had an anti-hyperglycemic

activity in a study employing a fructose supplementation-induced insulin resistance rat model. At oral dosages of 200 and 400 mg/kg body weight, respectively, the methanolic fruit extract of *L. acidissima* demonstrated an antidiabetic effect by lowering the glucose content by 19.2 and 27.5% in experimental rats.

In another study, diabetic rats' blood glucose levels on day 21 were lowered by a methanolic extract of *L. acidissima* Linn. fruit pulp at 200 and 400 mg/kg. In Wistar albino rats with Alloxan-induced diabetes, methanolic leaf extract of *L. acidissima* demonstrated antidiabetic and antioxidant activity through increased glycogenesis, improved glucose transport, and direct effects on islet regeneration or repair¹⁴.

Antioxidant Activity: Methanolic extract of dried rind and fruit pulp evaluated using the ascorbic acid as in percentage inhibition of ABTS free radical. The (IC₅₀ value) antioxidant activity as determined by ABTS assay and was found to be 0.7mg/ml and 0.8 mg/ml for methanolic extracts of the pulp and rind of kaitha, respectively. In FRAP assay, ferric-tripyridyltriazine reduced to form an intense blue coloured ferrous complex that can be measured at the 593 nm wavelength.

Intensity of the colour is related to the amount of antioxidant reductants present in the samples. As compared to the pulp (42.95 µg BHTE/ mg) (FRAP) Ferric Reducing Antioxidant Power assay activity was observed much higher in the rind (46.03 µg BHTE/ mg) of the fruit¹⁵. *Feronia limonia* significantly increased the superoxide dismutase and catalase activities which suggest *Limonia acidissima* may reduce free radical formation. *Limonia acidissima* also reduces total nitrite and MDA (methanol dialdehyde) which is lipid peroxidation marker which suggests its antioxidant activity¹⁶.

Neuroprotective Effect: Extract of Kaitha fruit obtained after extraction with methanol shows improvement in ischemia induced neurological status motor performance such as forelimb strength improvement, balance and coordination, grasping ability in ischemic rats¹⁶.

Antibacterial Activity: Three gram positive (*Staphylococcus aureus*, *Staphylococcus*

epidermidis, *Bacillus subtilis*) and a gram-negative bacterium (*Proteus mirabilis*) were used to evaluate the antibacterial activity of dried pulp and rind. Inhibiting concentrations used for both samples was 500 mg/ml.

The methanolic extracts of pulp were found to possess highest antibacterial activity against *Staphylococcus epidermidis* followed by *Staphylococcus aureus* and *Bacillus subtilis*. The rind of kaitha also revealed antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The antibacterial activity can be attributed to the phenolic content of the sample extracts.

The samples having higher phenolic contents were found to be better in inhibiting the growth of bacteria hence were giving zone of clearance of greater diameter. Further GC/MS studies clarifies that few hydrocarbons, sterols, aldehydes, carboxylic acids and their esters, phenolic acids, and flavonoids also contributing factors for antimicrobial properties of fruit extract¹⁵.

Anti-fertility Activity: Pulp of Wood apple remarkably reduces the testicular and epididymal protein content also glucose-6-phosphate dehydrogenase and Δ^5 - 3β -hydroxy steroid dehydrogenase level which shows improvement in sperm count, motility, viability and decrease percentage of abnormal sperm, whereas significant elevation was reported in the testicular Ascorbic acid and Cholesterol content. Pulp of fruit also found to protect against the oxidative damage and also plays a crucial role in spermatogenesis¹⁷.

Anti-cancer Activity: *L. acidissima* Linn fruit's extracts have reported to potent anticancer properties¹⁸. Ethanolic fruit fractions were utilised to ascertain the ED₅₀ value in two distinct breast cancer cell lines, SKBR3 and MDAMB-435, representing a 50% reduction of cancer cell growth. Ethanolic extraction of pulp at a concentration of 100 g/ml, reported to dramatically decrease cell growth in both cancer cells after 48 hours of treatment. Cell cycle examination of the fruit extract fraction in MDAMB-435 cells revealed that it caused an increase in the number of cells in the G2/M phase, however in SKBR3 cells, no

appreciable alteration in the cell cycle was reported¹⁹.

Wound Healing Properties: Studies on Rate experiments conducted using methanolic extracts of *Limonia acidissima* L. fruit pulp. Reported that when extracts were applied to the wound in the excision model, the wound gradually shrank and needed a mean of 16.0 +/- 0.8 days to heal properly. Incision wound models treated with MELA showed increased wound-breaking strength and shorter epithelization times²⁰.

***Limonia acidissima* in Fruit Industry:** *Limonia acidissima* fruit pulp is used for making food products like fruit bar, jam, pulp powder, wine, sherbet, chutney etc. *Limonia acidissima* jams are prepared from single fruit or from combination or two or more fruits^{9, 21}.

***L. acidissima* as Preservative:** The anthocyanin-rich fruit beverages and the anthocyanin that was extracted from fruits were found to be unstable due to chemical factors including pH changes, bleaching from preservatives and enzymes, and external processing parameters like light, temperature, etc.²¹ the different protein hydrolyzate concentrations that are taken from *L. acidissima* seeds in order to postpone the breakdown of the isolated anthocyanin from *Syzygium cumini* (L. The presence of a combination of different peptides may be the reason why the author proposed that a 2% concentration of *L. acidissima* protein hydrolyzate effectively delays the breakdown of anthocyanin²².

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CONFLICT OF INTEREST: Nil

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