

Pharmacological potential of wood apple (*Limonia acidissima*): A Review

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ABSTRACT

Wood apple is an edible fruit of Rutaceae family with less exploitation. The fruit pulp is edible and used in many food preparations around the globe. It is also used long time as traditional medicines. This review aimed to summarize the pharmacological potentials of this fruit. This fruit is effective against some deadly disease such as cancer, diabetes, hyperlipidemia and microbial infection. It also has capability to treat diarrhea, ulcer, and pain. Both ripe and unripe fruit has strong pharmacological activity. The presence of phenolic and flavonoids are the main antioxidant responsible for the therapeutic potentials.

Keywords: Antimicrobial, cancer, diabetes, pharmacological activity, wood apple

INTRODUCTION

Plants are one of the major source traditional medicines which have been used more than thousands of years ago. Natural plant based products has already gaining popularity because of its low side effects and its popularity is rising with widening range of applications from pharmaceuticals to cosmetics. The Medicinal & Aromatic Plants are main sources of different phytochemicals – alkaloids, phenolics, saponins, steroid, flavinoids, glucoside, terpenoids, tannins, aliphatic alcohols, acids and esters, etc. and essential oils with a wide variety of applications in pharmaceuticals, flavours and fragrance, disinfectants, oral hygiene and in almost all spheres of human activity (Sarwar, 2020). The fruit wood apple (*Limonia acissima* L) belongs to the family of Rutaceae and is found in India, Pakistan, Bangladesh, Sri Lanka and Southeast Asian countries (Rodrigues *et al.*, 2018; Hiwale, 2015). The fruit resembles an apple which leads to the establishments of the name. The peculiar fruit wood apple looks like a rotten coconut on the shell while in the inside it has soft pulp. The fruit smells like mixed stench of rotten blue cheese with overripe bananas. Wood apple pulp has sour and funky taste which is delicious with touch of sugar in desserts or with warm spices for savory dishes. The fruit is a remedy for cancer, diabetes, diarrhea, ulcer, and blood pressure. Regular consumption of this fruit helps to prevent from these disease.

PHYTOCHEMICALS AND NUTRIENTS

Wood apple contains phytochemicals like polyphenols, vitamins, saponins, coumarins, amino acids, tri-terpenoids, phytosterols and tannins (Pandey *et al.*, 2014). Phytochemical analysis of *Limonia acidissima* ripe fruits indicates presence of flavonoids, steroids, glycosides and various acidic compounds. The major chemical compounds in leaf are acidissimin and acidissiminol. Presence of alkaloids, phenolsresins, gum and mucilage, fixed oils and fats are also noted in leafs (Aneesha *et al.*, 2018; Panda *et al.*, 2013; Jayashree and Londonkar, 2014 and Vijayvargia *et al.*, 2014). The wood apple pulp is very good source of carbohydrates (70.14%), protein (13.8%), fat (4.3%) and dietary fibre (1.7%) (Pandey *et al.*, 2014; Asp, 1996). Presence of low amount of fat (4.38%), calcium, magnesium, iron, and high amounts of zinc are also reported in this fruit. High amount of phosphorous and calcium are also found out that exerts vital role in bone formation, blood clotting and more other metabolic processes (Table 1). The presence of iron in fruit indicates effectiveness against anemia, tuberculosis and other disorders (Campous *et al.*, 2009).

ETHNOMEDICINAL USES

Various parts of wood apple have been used for more than thousands of years in traditional medicines. Both ripe and unripe fruits have reputation for its medicinal properties. The phytochemical and minerals are responsible for

Table 1: Mineral and Vitamins content of kaitha pulp (Pandey *et al.*, 2014)

| Minerals (ig/g) | | | |
|-----------------------------|---------------------|---------------------------|---------------------|
| Analyte | Concentration(ig/g) | Analyte | Concentration(ig/g) |
| P | 1137.35 | Cr | 1.543 |
| Mg | 852.5 | Pb | 0.163 |
| Ca | 711.8 | Li | 0.241 |
| Fe | 23 | Mo | 0.263 |
| Zn | 23.84 | Ni | 0.819 |
| Cu | 6.67 | Se | 0.768 |
| Mn | 3.64 | Ti | 0.257 |
| Vitamins(ig/g) | | | |
| Vitamin C | 180 | Thiamine(B ₁) | 0.31 |
| Riboflavin(B ₂) | 0.23 | Beta-carotene | 0.04 |

providing therapeutic roles. In traditional system it is used to cure dysentery, diarrhea, asthma, wounds, tumors, hepatitis and cardiac debility. Ripe fruit also cure liver disease and heart problems. It has role on lowering cholesterol levels in blood (Vidhya and Narain, 2011 and Mishra and Garg, 2011). Juice of wood apple (50mg/ L) with warm water and sugar is recommended for detoxification via blood purification and removal of toxins from the body (Vasant and Narasimhacharyaa, 2011). In children, juice of wood apple leaves mixed with milk and sugar is given for the remedy of biliousness and intestinal problems. The essential oil of crushed leaves is used to cure itching and improves digestion problem (Morton, 1987). Wood apple leaves contains high amounts of tannins which is effective against peptic ulcer. The leaves are also effective in the treatment of breast cancer, uterus cancer, infertility, progesterone deficiency, flu and respiratory disorders (Jayakumar and Geetha, 2012). The leaves have traditional use in snake bites and against bacterial pathogens (Kirtikar and Basu, 1995). Astringent, carminative and hepatoprotective activity are also known for leaves (Ilango and Chitra 2009). The pulp has low fat content (4.38%) and is an effective diet for overweight people (Pandey *et al.*, 2014). The fruits are refrigerant, stomachic, stimulant, diuretic, astringent, aphrodisiac, cardiac tonic, liver tonic, anti-asthmatic, antidiarrheal, leucorrhoeal and also effective against dysentery. The seeds are used in

the treatment of heart diseases (Jadeja *et al.*, 2005; Senthilkumar *et al.*, 2010). Spine of the tree is a remedy of menorrhagia. The bark contains 0.016% marmesin, aurapten, bergapten and other coumarins and is applied on venomous wounds upon crushing (Morton, 1987). The gum has demulcent, constipating, anti-diarrheal and anti-haemorrhoidal properties (Jayakumar and Geetha, 2012).

PHARMACOLOGICAL PROPERTIES

Anticancer activity

Essential oils isolated from leaves of wood apple shows antioxidant and cytotoxic activities. Thirugnanasampandan and David (2014) demonstrated that essential oil of 89.19µg/ml inhibits human cancer cell line MCF-7 (Michigan Cancer Foundation-7) by DNA fragmentation. In the same ways Pradhan *et al.* (2012) evaluates antineoplastic activity of ethanolic extracts of fruits on human breast cancer cell lines (SRBR3 and MDA-MBA435) and found effective dose ED50 of 56.1 and 30.6ig/ml respectively. Eluru *et al.* (2015) reveals the in vitro anti-tumor activity of methanolic extracts of fruits at oral dose of 570mg/ kg body weight on mice model of Dalton's Ascitic Lymphoma (DAL) cell found that treatment with extract enhance nonviable cell counts in peritoneal exudates and decrease the viable cell count which may be because of absorption of extract by viable cells and the ultimate results was cell lysis by

activation of macrophage or any type of cytokine production. The extract also restored RBC and hemoglobin content (Eluru *et al.*, 2015). The anticancer property of the fruits is may be due to the presence of flavonoids (Ilango, and Chitra, 2009).

Antidiabetic activity

Anti-diabetic activity of 95% ethanolic extracts of unripe wood apple fruits was evaluated on streptozotocin-induced diabetic rats at 250mg/kg body weight and found that it considerably lowers blood glucose levels of fasted, fed, and streptozotocin-induced diabetic rats (Gupta *et al.*, 2009). Hypoglycemic effects are also observed on alloxan-induced diabetic rats. Methanolic extract at 1.75g/kg body weight is effective in prevention of hyperglycemia (Mishra and Garg, 2011). A 21 day trial of wood apple bark at dose of 200mg/kg and 400 mg/kg body weight of methanolic extract reduces blood glucose level by 39% and 54.5% respectively (MohanaPriya *et al.*, 2012). A similar effect was found by alloxan induced rat which was measured by blood serum levels (Ilango and Chitra, 2010). Anitha *et al.* (2015) also determined significant lowering of fasting and post prandial blood sugar level after consumption of fruit juice for 90 days.

Antihyperlipidemic activity

After administrating fruit powder at 2.5, 5 and 10 g/kg body weight for 28 days reduces lipid profile, hepatic glucose-6-phosphatase, and significant increases hepatic glycogen, hexokinase and HDL. The presence of fibres, phytosterols, saponins, polyphenols, flavonoids and ascorbic acid may be responsible for that (Rupal *et al.*, 2013).

Antioxidant activities

Antioxidant activity of wood apple pulp was determined by water, petroleum ether, chloroform, ethyl acetate, and methanol extracts by Priya Darsini *et al.* (2013). They conducted 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, trolox equivalent antioxidant capacity (TEAC) assay, hydroxyl radical scavenging assay (HRSA), ferric reducing antioxidant power (FRAP) assay, nitric oxide radical (NO) scavenging assay, and total antioxidant activity (TAA) and found marked

antioxidant activity. In-vitro antioxidant activity of Petroleum ether, chloroform and methanolic extract of wood apple leaf was determined by DPPH radical scavenging activity, Nitric oxide radical scavenging activity and hydrogen peroxide scavenging activity method and reveals that all the extract possesses sufficient antioxidant property (Attarde, 2016). Similar activity was also reported by Kerkar *et al.* (2020) and Singhania *et al.* (2020). Methanolic extract was also effective in synergizing antioxidant enzymes such as superoxidase dismutase and catalase (Patel *et al.* 2012). A 30 days oral administration of ethanolic extract of leaf at 200mg/kg body weight was effective in increasing activity of enzymatic antioxidants like Glutathione, superoxide dismutase (SOD), catalase (CAT) and peroxidase (Balamuruganvelu *et al.*, 2015).

Hepatoprotective activities

Hepatoprotective activity of methanolic extracts of pulps was evaluated by Ilango and Chitra (2009) against carbon tetrachloride (CCl₄)-induced liver damage in rats. They found that levels of hepatic enzymes especially aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AST), total protein (TP), total bilirubin (TB), and gamma glutamyltransferase (GGT) was increased and levels of super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) decreased by treatment of 100, 200 and 400mg/kg i.p. for 3 days in rats. In another study aqueous leaf extract raises serum alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) level. It also elevates urea, creatinine, potassium and sodium levels in the phenyl hydrazine-induced anemic non-treated rats compared to the normal control (Anacletus *et al.*, 2019).

Diuretic activity

The methanolic extract of leaves has significantly increase urine output. The extent of urination and electrolyte excretion namely Sodium, potassium and chloride ions depend on extraction method. It was found that Microwave assisted extraction (MAE) has better activity as compared to Bath Sonicator extraction (BSE)(Parial *et al.*, 2009).

Antiulcer and wound healing activities

It was found that that wood apple fruit pulp is effective against indomethacin-induced gastric ulcer in rats. At 500mg/kg it inhibits gastric ulceration by reducing gastric HCl concentration through increasing intra-gastric pH (Mishra *et al.*, 2009). Wound healing activity of methanolic fruit extract increased by tightening wound-breaking strength, decreased epithelization period, increased wound contraction, and increased granulation tissue weight and hydroxyproline concentration at 400 mg/kg of the extract (Ilango and Chitra, 2010). *L. acidissima* extracts significantly protect the gastric mucosa against ethanol induced injury by the reduction in the mucosal lesions on dose dependent manner. The phenolic compounds presents in the fruit is responsible for protection against ulcer on gastric wall and leucocytes infiltration of submucosal layers at 400 mg/kg leaf extract (Aneesha *et al.*, 2018).

Analgesic activity

The analgesic activity was found against acetic acid- induced writhing mice and found 60.53% on methanol, 59.65% on acetone extracts of fruit peel as against 78.07% on standard drug Diclofenac Na (Islam *et al.*, 2020).

Neuroprotective activities

Neuroprotective effects of wood apple was evaluated and showed that at 250 mg/kg and 500mg/kg body weight it inhibits ischemia reperfusion-induced brain injury in rats (Rakhunde *et al.*, 2014).

Spermatotoxic activities

Dhanapal *et al.* (2012) studied antispermatogenic activities of wood apple fruit pulp in adult male rats by treating with ethanolic extracts at 250 and 500mg/kg for 55 days and reveals that they were responsible for decline in sperm count, motility and viability. They also increased proportion of abnormal sperm and reduce testicular protein content by 24.58% and 29.86%, respectively.

Antidiarrheal activity

Senthilkumar *et al.* (2010) determined antidiarrhoeal and gastrointestinal motility reducing

activity on aqueous bark extract of wood apple and found marked antidiarrheal activity by reducing average faeces weight and reduce GI motility (Senthilkumar *et al.*, 2010). Similar data was found by Thomas method on castor oil-induced diarrhea at 500mg/kg of methanol and acetone peel extracts and observed 47.13% and 44.83% inhibition (Islam *et al.*, 2020).

Antimicrobial activity

The methanol and acetone fruit peels extract at 250mg/kg shows moderate activity which inhibits 34.45% and 35.63% on *Klebsiella Oxytoca*, *Vibrio metschnikovii*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Islam *et al.*, 2020). The essential oil present on wood apple leaves containing β -pinene (28.4%), Z-anethole (22.1%), methyl chavicol (12.0%), and E-anethole (8.1%) which exerts antibacterial activity against five Gram-positive (*Staphylococcus aureus*, *Micrococcus flavus*, *M. luteus*, *Bacillus subtilis*, *Streptococcus faecalis*) and eight Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Serratiamarcescens*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterobacteraerogenes*), and four fungi (*Aspergillusniger*, *A. fumigatus*, *Penicilliumchrysogenum*, *Candida albicans*). The oils are also effective against *Micrococcus luteus*, *Proteus mirabilis*, *Penicillium chrysogenum*, and *Aspergillus niger* with minimum inhibitory concentration values of 0.31, 0.52, 0.20, and 0.26 mg/ml, respectively (Joshi *et al.* 2011). Antibacterial activity was also assessed by agar well diffusion method against three gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*) and a gram negative bacterium (*Proteus mirabilis*) (Pandey *et al.*, 2014). The essential oils are present in different parts of the plant and provides antibacterial activity (Bagul *et al.*, 2019). Minimum inhibitory concentration (MIC) was also determined by Naidu *et al.* (2014) on leaf extract in hexane, chloroform and methanol and show sufficient activities. Biosynthesized silver and zinc oxide nanoparticles was tested against *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* and found

effectiveness (Patil and Taranath, 2018). Antimicrobial activity was also determined by well diffusion method against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Sonawane *et al.*, 2018). There is another study of silver and zinc oxide nanoparticles on *S. aureus*, *S. typhi* and *P. aeruginosa* at 400 µg/ml and found maximum zone of inhibition 15.16, 15.5 and 13.33/ mm respectively. Although zinc oxide Nano particles shows less activity as by comparison with silver Nano particles (Bheemanagouda *et al.*, 2018). These Nano particles are also effective against *M. tuberculosis* at 12.5 ig/ml (Bheemanagouda *et al.*, 2016). The wood apple is used traditionally in Thailand to treat oral and throat infection (Lairungruang *et al.*, 2014). Pandey *et al.* (2014) evaluates antibacterial activity of dried pulp and rind at 500 mg/ml on *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Proteus mirabilis*. Other researchers are also reported antibacterial activity of wood apple (Panda *et al.*, 2013; Momin *et al.*, 2013 and Naidu *et al.*, 2014).

Anti-fungal activity

Pradhan *et al.* (2012) claims that fruit shells of wood apple has antifungal compounds such as psoralene, xanthotoxin, 2, 6-dimethoxy benzoquinone, and ostenol. Antifungal activity of leaf ethanolic extracts was done by cup plate techniques on *M. gypseum*, *T. tonsurans*, *T. mentagrophytes*, *C. albicans* and least *T. rubrum* in dose dependent manner (Shivakumar and Vidyasagar, 2015).

Larvicidal activities

Wood apple leaf is effective against larvae of *Culex quinquefasciatus* with 90% mortality at 3% aqueous extract. The chloroform and methanol extract also shows 95% mortality at 100 ppm concentration (Banerjee *et al.*, 2011). Mosquitocidal activity was found due to the presence of terpene in wood apple leaf which inhibits eggs, larvae, and pupae of *Aedes aegypti*. The extracts have very good larvicidal and pupicidal activity (Reegan *et al.*, 2014). Hexane extract of *L. acidissima* shows ovicidal activity 79.2% and 60% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. Aegypti* (Reegan, *et al.*, 2015).

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