

Medicinal Uses and Pharmacological Activity Wood Apple (*Limonia Acidissima*) : A Review

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ABSTRACT

A fruit of the Rutaceae family with less commercial use is the wood apple. Food preparations all throughout the world use the fruit pulp, which is edible. It has also been used for centuries as a traditional medicine. Although they are not frequently used, seeds from fruits like *Citrullus* (C.) *lanatus* (watermelon) and *Limonia* (L.) *acidissima* (wood apple) could be useful in many food formulations. It was determined that defatted seed flours had protein contents of 71.38 and 49.51 percent, respectively, and that they also included significant amounts of minerals like Na, Mn, Mg, K, Cu, Fe, and Zn. the reduced L. The goal of this review was to provide an overview of the fruit's pharmacological potential. This fruit is useful against a number of dangerous diseases, including bacterial infections, cancer, diabetes, and hyperlipidemia. pH, total soluble solids, titratable acidity, ascorbic acid, and biochemical characteristics (total phenolic content and antioxidant activity) of the chutney were examined during the storage period. One of the native milk products is kalakand, which is made by heating whole or standardised milk and then concentrating it with the addition of sugar and the right coagulant. The goal of the current experiment was to create Kalakand by incorporating wood apple pulp into it at various concentrations while using buffalo milk. Additionally, it has the ability to treat pain, ulcers, and diarrhoea. Fruit's pharmacological action is significant in both ripe and unripe forms. The principal antioxidant responsible for the medicinal potentials is flavonoids and phenolic compounds.

Keywords: Wood Apple; *Limonia Acidissima*



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INTRODUCTION

Wood apple (*Limoniaacidissima*) Fruit is a commonly found member of the Rutaceae family and is an underappreciated fruit. Elephant apple, monkey apple, Kotha, kainth, and other names are also used to refer to it in different regions of India. It is typically found throughout the plains in states like Maharashtra, West Bengal, Chhattisgarh, Uttar Pradesh, and Madhya Pradesh. It is also found in the western Himalayas. [1, 3]The fruit's brown, extremely hard outer shell contrasts with the pulp's sour flavour and imbedded seeds. The amount of flavonoids, glycosides, saponins, and tannins in wood apples is sufficient. [4, 5]furthermore a rich source of ascorbic acid, riboflavin, vitamin B complex, beta-carotene, and minerals, with seeds having a high oil content [6].The fresh wood apple has 7.1 grammas of protein, 18.1 grammas of carbohydrates, 10.45-21.70 percent TSS, 1.98- 3.80 percent treatable acidity, 4.77-5.71 TSS/acid ratio, 0.30-6.03 percent reducing sugars, 5.65-13.80 percent non-reducing sugars, 7.95-19.83 percent total sugars, 3.86-6.82 mg/100g ascorbic acid, 221.50- 80.10 mg/100g total phenol, and 9.88 [7,9].Although having beneficial nutritional and medicinal qualities as well as a delicate flavour, this fruit is currently neglected. Dysentery and other infections are treated with wood apple in traditional medicine. Fruit contains photochemical that have hepatoprotective, antifungal, hypoglycemic, and anti-oxidative characteristics, as well as hypolipidemic and hypoglycemia effects. [11, 12]Different parts of wood apple plant have been reported to exhilarated liver tissue, cut injuries, skin malignant growth, bosom disease and cell reinforcement exercises [13]Wood apple fruit, which ripens to become soft, is used to make fruit bars, drinks, sweets, and fruit smash, which is used to make jam and ready-to-serve beverages. Fruit pulp is a useful ingredient in blended drinks made with coconut milk and palm sugar syrup. This fruit is still underutilized, not in demand, and has no market value in spite of its various nutritional and health benefits. As a result, an effort was made to

turn the fruit of the wood apple tree into a value-added product and to research its various physicochemical characteristics. [14, 15] According to the Rome IV Criteria, the sixth most frequently reported gastrointestinal (GI) disease is constipation, which is defined as the inability to pass less than 35 g of stools per day, less than 3 bowel movements in 7 days, a faecal water weight of less than 70%, and a gastrointestinal (GI) transit time of more than 5 days. [16, 17, 18] This will not only create new opportunities for maximising the use of nutritious fruits with large yields, but it will also encourage the growth of the wood apple processing business. Since consumers have the final say in what food is chosen, research on acceptability and nutrients is crucial. [19] It grows naturally in the dry plains of India, Pakistan, and Sri Lanka where it is also occasionally grown in orchards, along roads, and on the edges of fields. Additionally, it is grown all over Southeast Asia, particularly in Malaysia. The fruit was traditionally considered a "poor man's food" in India until processing methods were created in the middle of the 1950s. In addition to being known as "Wood apple," other names for it include "elephant apple," "monkey fruit," "curd apple," "Kethbel," "golden apple," "stone apple," etc. It is referred to as *gelinggai* or *belinggai* in Malaysia, *ma-khwit* in Thailand, *kramsang* in Cambodia, and *ma-fit* in Laos. It is known as *pommel' elephant*, *pommel de bois*, or *citrondesmois* in French. This well-known fruit contains a number of well-known and one of the most valuable medicinal plants in India due to its medicinal characteristics. The conversion of complex sugar into simple sugar during the wood apple product's storage period raises the product's overall solid soluble content. The data shows that the pH of the chutney dramatically reduced during the course of the storage time as a result of the wood apple chutney's increased acidity. The outcome Husaini et al. reported. [20] This level of acidity in mango chutney denotes pH fall brought on by increases in acidic ingredient over extended storage. Ascorbic acid was measured at 4.2 mg per 100 grammes on week zero and 2.2 mg per 100 grammes on the last week of storage. It was consistently reduced throughout the storage period as a result of ascorbic acid reacting with trapped oxygen in the glass bottle to produce highly volatile and unstable dehydrate ascorbic acid, which then degraded to ketogulconic acid or furfural compounds as a result [21,23]. Have been observed that ascorbic acid levels in squash made from wood apple fruit have gradually decreased. On the first day of storage, ascorbic acid levels in preserved wood apple reached 0.44 mg/100g. The amount of total phenolics in wood apple chutney dramatically declined during the course of storage; on the first day, it was 190.2 mg GAE/g, and by the last day, it had dropped to 143.7 mg GAE/g. According to Deng et al. [24], over the time that the litchi pericarp was stored, the phenolic content decreased gradually at first, but subsequently rapidly as a result of greater enzyme activity in the pericarp, which hastened the oxidation process. Furthermore, Joshi et al. [25] In India, fruit is frequently used as a liver and heart tonic. It is used to treat diarrhea and dysentery when it is unripe. In addition, wood apple exhibits hypoglycemic, anti-hyperlipidemia, wound-healing, anticancer, diuretic, larvicidal, and antimicrobial activity, as well as hepatoprotective, anti-inflammatory, antipyretic, and analgesic properties (Vidhya and Narain, 2011). [26] Since wood apples are a seasonal and highly perishable fruit, they can be preserved by being processed into a variety of value-added goods, such as chutney, fruit bars, RTS, squash, nectar, pickles, jam, and pulp powder, so they can be used all year long. The fruit can be consumed raw, but because of its resinous flavour, it must be sweetened. The pulp of the ripe fruit is typically used to make delicious chutney, though it can also be eaten straight away after being sweetened. (2015) Anuradha K. [27] Due to their great nutritional value, wood apple fruit is widely renowned for its medicinal capabilities. 1980's Geda and Bokadia [28] observed the essential oil from wood apple fruits' antibacterial properties and noted that it was effective against 12 different human pathogenic microorganisms. (2000) Maiti and Mishra [29] have observed that the fruits of wood apple trees exhibit antivenom action. Hiccups can be treated with wood apple, which can be found in chutneys and sherbet. Research has shown that the wood apple's fruits, leaves, and stem bark have anti-tumor and antibacterial properties. Antioxidant and diabetic properties of wood apple. According to Patel et al. (2012), it works by lowering blood glucose and malondialdehyde levels. [30] Cathartics, laxatives, and purgatives are all included within the category of anti-constipation medications. While all three classes strive to increase defecation, their levels of action are different. In contrast to laxatives, which function more slowly to treat acute non-dietary constipation, purgatives have a greater action that causes more fluid to be evacuated from the rectum. Laxatives are therefore milder than purgatives. Even more potent than the two are cathartics that encourage colonic evacuation. Cathartics are therefore the most potent, followed by purgatives and laxatives. [31] Laxatives are composed of chemicals to increase stool motility frequency. [32] Due to their stimulatory action to defecate, which is brought about by enhanced water and electrolyte transport into the gut mucosa while also softening hardened feces, they are the most frequently recommended class of medications for constipation. Since most laxatives are available over-the-counter, they are frequently bought to treat constipation on one's own, which results in low drug compliance and effectiveness. [33, 34]



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PLANT DESCRIPTION

An average tree can reach a height of 12 metres (40 ft). Even though it grows slowly and is a subtropical species, the tree can withstand a wide temperature range, from -6°C to 48.8°C . This plant is reproduced through seed. Almost any sort of soil will support the growth of the wood apple. It can easily survive extended droughts. Once established, it requires little maintenance. Seven years are needed for saplings to start bearing fruit. After flowering, the fruit takes 10 to 12 months to ripen.

VARIETIES

Two of the several wood apple kinds that are grown in India are highly well-liked: the yellow bael, which tastes sweet when ripe, and the kodbael, which is both sweet and sour. Both are quite beneficial medically. This fruit and its plant are the subject of extensive research by numerous pharmaceutical companies.

SEASON

In Malaysia, flowering takes place in February and March, the fruit ripens in October and November, and the leaves are lost in January. The fruit ripens in India between early October and March.

DISTRIBUTION

India, Sri Lanka, Pakistan, Java, and Malesia have all reported finding *Limonia Acidissima* L. on a global scale. It is seen to happen in the desert regions of India. The states that have reported it are Punjab, Delhi, Rajasthan, Madhya Pradesh, West Bengal, Arunachal Pradesh, Maharashtra, Goa, Karnataka, Tamil Nadu, and Andhra Pradesh. This species is widely distributed throughout Indo-Malesia. All over the Indian plains, especially in the drier sections, it is cultivated for its fruits. [35]"

MORPHOLOGICAL CHARACTERISTICS:

The *Limonia Acidissima* L. tree grows to a sizeable, deciduous, upright tree in India. It has a few rising upward branches that bend outward near the summit, where they are divided into thin, drooping branchlets."[36]" It is a slow-growing tree with a 9 m height that grows well in desert, warm areas up to 450 m elevation and frequently has rough, thorny bark. It is found throughout India. The spines are axillary, tiny, straight, and 2–5 cm long on a few of the zigzag twigs.

DESCRIPTION OF DIFFERENT PARTS OF LIMONIA ACIDISSIMAL

Limonia Acidissima L. has alternate, deciduous, three to five-inch-long, dark green, leathery leaves. Born in small, loose panicles that are terminal or lateral, frequently minutely serrated, blunt, or notched, dull crimson, or greenish. The fruit is round to oval in shape and is 5-12.5 cm across. It has a woody, extremely hard peel that can be difficult to break. The fruit is greyish-white in appearance and has a scurfy peel that is around 6 mm thick. The pulp of *Limonia Acidissima* L. is black, aromatic, and gooey. It is fragrant, resinous, astringent, acidic, or sweet-tasting and has numerous tiny, white seeds strewn throughout it. There are two different types, one with large, sweet fruits and the other with small, tart ones.

LimoniaAcidissima L. is a plant that has a pleasant scent. The 6 mm-thick, grayish-white rind has a rough texture. It has a woody, extremely hard exterior shell called the rind that is exceptionally difficult to crack open. The hard rind of the wood-apple fruit is broken with a hammer.[37]”

MEDICINAL USES:

- ❖ *LimoniaAcidissima* L. is commonly used as a liver and heart tonic in India. To treat poisonous insect bites and stings, the pulp is used as a poultice.
- ❖ Successful treatments for diarrhoea, dysentery, a sore throat, gum infections, and a cough include *LimoniaAcidissima* L.
- ❖ Traditionally, the stem bark of *Limoniaacidissima* is mixed with water and applied largely on the face (Yoganarasimhan, 2000). Regular application to the skin is supposed to keep it cool, smooth, fair, and well-textured, according to Patra, Mishra, and Chaudhuri (1988). It is also known to prevent skin cancer by blocking UV light.
- ❖ Minor skin lesions and spots are treated using the facial cosmetic "Thanaka," which is made from the pulp of *L. acidissima* (Bandara et al..[38]

PHARMACOLOGICAL ACTIVITY:

Wound Healing-

Using a methanol extract of *LimoniaAcidissima* L. fruit pulp as a screening agent, albino rates of either sex were utilised to evaluate the wound healing ability. When the extracts were applied to the wound in the excision wound model, the wound gradually shrank and needed a mean healing time of 16.0 +/- 0.8 days. Incision wound models treated with MELA (Methanol extract of fruit pulp) had higher wound breaking strength and shorter epithelization times. Different *LimoniaAcidissima* L. extracts have potent, dose-dependent wound-healing properties.”[39]”

Antioxidant Activity-

Antioxidant activity was tested in a crude methanol extract of *Limoniaacidissima* L. stem bark "[40]". On the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH), the antioxidant activity of the partitionates was assessed. The crude methanol extract's chloroform soluble fraction (CL) has the greatest capacity to scavenge free radicals. The pet ether soluble fraction (PE) also showed significant antioxidant activity at the same time. By using the FRAP and DPPH radical scavenging assays, the methanol extract of *LimoniaAcidissima* L. fruit was also examined for its capacity to scavenge free radicals. Different *Limoniaacidissima* L. leaf extracts have been studied and shown to have in vitro antioxidant activity. [41-43].

Anti-bacterial Activity-

By using the agar well diffusion method, the antibacterial activity was assessed against Gram-negative and Gram-positive bacteria. Hexane extract was found to be less active, whereas chloroform extract displayed mild to moderate activity and methanol extract shown good antibacterial activity with substantial inhibition zones. [44]Using the disc diffusion method, the antimicrobial activity of methanol extracts from *LimoniaAcidissima* L. plant parts was evaluated against *Escherichia coli* and *Staphylococcus aureus*. Different parts' extracts displayed varying levels of anti-microbial activity. In general, plant extracts from every section of the plant efficiently restrained the development of both gramme positive and gramme negative bacteria. The pulp extract from *L. acidissima*'s 5 investigated parts (bark, leaf, shell, pulp, and seed) had the strongest inhibitory impact on both bacteria. “[45]”.

To test the anti-bacterial effectiveness of dried pulp and rind, three gramme positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) and one gramme negative (*Proteus mirabilis*) bacteria were utilised. It was discovered that the pulp's methanol extract had the strongest antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus subtilis*. Along with having antibacterial properties against *Staphylococcus aureus* and *Staphylococcus epidermidis*, *LimoniaAcidissima* L.'s rind also has “[46]”.

Anti-fungal Activity-

The various extracts of *limoniaacidissima* L. fruit pulp showed antifungal efficacy against several pathogenic fungi (petroleum ether, chloroform, methanol, and aqueous). [47]”

EFFECT OF ACTIVE CONSTITUENTS:

1) **Flavonoids** - Polyphenolic substances called flavonoids are found in nature. Flavonoids are secondary plant metabolites that have antioxidant properties and share the chromane ring with tocopherols. Iron chelation, direct scavenging of oxygen and nitrogen free radicals, suppression of oxygen radical-producing enzymes, and reduction of leukocyte adherence to the blood vessel wall during tissue inflammation and reperfusion are the main mechanisms involved in this action. Flavonoids play a key role in the ageing process of the skin. By preventing extracellular matrix-degrading enzymes like collagenase, elastases, and hyaluronidases from breaking down the extracellular matrix,

flavonoids like kaempferol postpone skin ageing. “[48]”. The best antioxidant activity and rancidity protection come from flavonoids. The antioxidant action of *Limonia Acidissima* L. is due to the presence of ascorbic acid in its pulp. Additionally, flavonoids have anti-fungal, anti-bacterial, and anti-microbial properties. According to reports, quercetin totally prevents *Staphylococcus aureus* from growing. “[49]”.

2) **Tannins** In addition to proteins, polysaccharides, alkaloids, nucleic acids, and minerals, tannins are a heterogeneous collection of high molecular weight polyphenolic chemicals. Gallotannins, ellagitannins, complex tannins, and condensed tannins are the four types of tannins that are separated based on their structural makeup. In 1905, (Maximilian Nierenstein) investigated the natural tannins present in many plant species. “[50]”.

Cosmetics-

- Precipitate protein, found in tannin, is used to treat burns and protect irritated skin surfaces.
- Tannins have antibacterial, antioxidant, and astringent properties.
- Tannins delay the ageing process of the skin.
- Tannins also function to stop hair loss. [51]

3) Saponins

Saponins are bioactive substances that are mostly made by plants. Chemically, they exist as polycyclic triterpenes or steroid glycosides. They can interact with cell membranes and lower the surface tension of an aqueous solution due to their lyobipolar characteristics. The name "saponin," which is derived from the Latin word "sapo" and refers to the creation of stable soap-like foam in aqueous solution, derives from this process. “[52]”

Cosmetics-

- Saponins are known as natural surfactants; in aqueous solutions like soap, they produce stable foam.
- They serve as a foaming agent in toothpaste, liquid detergent, and shampoo.
- Emulsifiers and long-lasting foaming agents both use saponins.
- Cosmetic products with saponin extract as an active ingredient have anti-oxidant, anti-aging, and regenerative qualities. “[53]”.

4) Alkaloids

The term "alkaline" was once used to denote any base that contained nitrogen, and this is where the name "alkaloids" comes from. They are typically organic bases that combine with acids to form salts, which, when soluble, result in alkaline solutions. Alkaloids are a class of chemical substances that exist in nature and primarily include basic nitrogen atoms. Some related compounds with neutral or even mildly acidic properties are also a part of this group. “[54]”

Cosmetics-

- Pyridine alkaloids have been found to present strong antimicrobial properties and have antioxidant activities because of their capacity to act as free radical scavengers, hydrogen donors, or electron or metal chelating activity"[55].
- Alkaloids are responsible for anti-bacterial and anti-fungal activity.
- Alkaloids provide a warming effect that can be applied, for example, to feet.
- Alkaloids combat wrinkles.
- Alkaloids also help in Skin-tightening” [56]”.

1. PH

Utilising a digital pH metre that was standardised with buffer solutions of 4.0 and 7.0, fresh fruit's PH was measured [57]. Fresh fruit was smashed using a pestle motor to determine pH, and the resulting solution was utilised to assess pH.

2. Visual color

A digital chromometer (Konica Minolta R-400/410 digital chromometer) was used to measure the visual colour of fresh fruit, tray-dried powder, and lyophilized powder.) [58].

3. Total soluble solids

Using a handheld refractometer (0-90 0Brix), the total soluble solids (TSS) of fresh fruits and processed goods were calculated at room temperature (25 oC). Results of a three-fold reading were represented as Brix (0Brix) degrees.

4. Treatable acidity

Fresh fruit's treatable acidity was assessed by placing a three-gram sample in a beaker with 100 ml of distilled water and heating it for an hour. After cooling, filtering was done, and 500 ml of distilled water was used to prepare the final

volume. A 5 ml aliquot of the extract and a few drops of phenolphthalein indicator were then put to a 50 ml conical flask. The titer value was recorded when this was titrated against 0.1 N NaOH until a faint pink colour persisted for 30 seconds. The average of three measurements of the acidity that can be treated was reported as percent citric acid.

5. Moisture content

Five grammes of the sample (pulp, seeds, and whole fruit) were placed in clean, pre-weighed petri plates before being dried in a hot air oven at 100°C for 5–6 hours, or until a constant weight was achieved. Weight of petri plates and samples were recorded for calculation after chilling in the desiccator.

6. Ash content

Five grammes of the sample (pulp, seeds, and entire fruit) were placed in weighted dried silica crucibles and heated on a hot plate until the sample turned black or the smoke stopped. The charred sample was then placed in a muffle furnace that had been preheated to 550 °C, where it remained for 4–5 hours or until white coloured ash was formed. In a desiccator, crucibles were cooled, and weight was recorded.

7. Total Ascorbic Acid

To extract ascorbic acid, two grammes of each sample were placed in a conical flask and mixed with 20 millilitres of a meta-phosphoric-acetic acid solution. The mixture was transferred to a volumetric flask after being filtered using Whatman filter paper. In a hurry, 2, 6 dichlorophenol indo-phenol solution was titrated with 2 ml of the filtrate extract and 5 ml of meta-phosphoricacetic acid solution until a light, definite rose-pink colour remained for more than 5 seconds.

8. Reducing sugar

The approach proposed by Ranganna was used to estimate the total amount of reducing sugars in the sample using Fehling's 'A' and 'B' solutions.

9. Microbial growth

Using a malt extract medium and the conventional plate count method, the microbial contamination and growth were assessed. After 48 hours of incubation at 38° C with sterile distilled water as the control, the plate was counted. [59].

10. Total Phenolic content

The Folin-Ciocalteu test method by UV vis mass spectrophotometer was used to assess the total phenolic content of the fruit sample extracts. In a volumetric flask (25 ml), the reaction mixture of 1 ml of extract and 9 ml of distilled water was taken. The mixture was treated with one millilitre of the Folin-Ciocalteu phenol reagent by vigorously shaking. The mixture was treated with 10 ml of a 7% sodium carbonate (Na₂CO₃) solution after five minutes. Deionized water was then added to the mixture to make a total volume of 25 ml, and a spectrophotometer was used to measure the absorbance of the test and standard solutions in comparison to the reagent blank at 650 nm. [60].

Pharmacological Effects

Wood apple fruit has several pharmacological effects that are attributed to its chemical constituents. Some of the main pharmacological effects of wood apple fruit include:

1. **Antioxidant:** The wood apple fruit has strong antioxidant capabilities due to its high concentrations of tannins, flavonoids, and vitamin C. Cells are helped by antioxidants to resist oxidative stress and damage brought on by free radicals.
2. **Anti-inflammatory:** The wood apple fruit includes a number of polyphenols that have anti-inflammatory characteristics, including gallic acid and ellagic acid. These substances might aid in reducing bodily inflammation, which can be a factor in a number of chronic disorders.
3. **Anti-diabetic:** Research suggests that wood apple fruit may improve insulin sensitivity and lower blood glucose levels, which may have anti-diabetic properties.
4. **Anti-cancer:** According to numerous studies, the wood apple fruit's polyphenols, particularly ellagic acid, offer anti-cancer qualities. It has been demonstrated that these substances prevent the growth and division of cancer cells.
5. **Immunomodulatory:** Wood apple fruit has been demonstrated to have immunomodulatory properties, which means it may aid to control and enhance the immune system's performance.
6. **Antimicrobial:** Research on wood apple fruit has revealed that it is particularly effective against bacterial strains including *Staphylococcus aureus* and *Escherichia coli*.
7. **Gastroprotective:** The wood apple fruit is gastroprotective, which means that it can help to protect the stomach lining and lower the risk of ulcers. This is due to the high quantities of tannins in the fruit.

Overall, the pharmacological effects of wood apple fruit are attributed to its rich chemical composition, which makes it a potent natural remedy for various ailments. However, more research is needed to fully understand the mechanisms of action and potential clinical applications of wood apple fruit. [61-65]

Conclusion

According to the current study, defatted *C. lanatus* and *L. acidissima* seed flours are good sources of protein and other nutrients, like minerals. These features are crucial and imply that these flours would be suitable for use as a matrix or as an ingredient in other food fortification preparations. Given the excellent functional characteristics of these flours, this is extremely likely. The defatted flours' thermal stability, spherical morphology, and amorphous nature will be advantageous for food system development or inclusion. According to the results of the current study, wood apple chutney is superior in terms of bioactive compounds like phenolic content and its antioxidant capabilities, which increase the product's quality and create a substantial opportunity for the marketing of goods made from the underutilised fruit. The purpose of the study was to ascertain how the storage period of wood apple chutney affected its physicochemical properties, total phenolic content, and antioxidant activity. The results of the study suggest that consumers accepted chutney based on its sensory qualities and that physicochemical properties degraded over time. During the storage period, a gradual decrease in the value of total phenolic content and antioxidant activity was seen. Learn more about improved atmospheric packing that improves or keeps the functional qualities throughout storage.

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