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Traditional, Pharmacological, and Therapeutic Properties of *Tamarindus indica*

Review Article

Radha S* and Kusum S

Department of Zoology, Institute of Basic Sciences, Bundelkhand University, Jhansi- 284128 (UP), India

***Corresponding author:** Radha Singh, Department of Zoology, Institute of Basic Sciences, Bundelkhand University, Jhansi- 284128 (UP), India, Email: radha060291@gmail.com

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Abstract

Plant materials have been used traditionally as medicine for treating ailments and retaining health. *Tamarindus indica* is one of the reported ancient herbal medicine plants. Tamarind is a species of the Leguminosae family (subfamily: Caesalpiniaceae). It is an evergreen tree, native to Africa and, at present, widely distributed in other tropical and subtropical parts of the world. In Ayurvedic literature, the pharmacological properties of tamarind are responsible for therapeutic efficiency. It is used as a flavoring agent in various dishes and beverages. Tamarind fruit is rich in polyphenols and flavonoids and shows a moderate antioxidant effect. Tamarind is a rich source of essential amino acids, phytochemicals, and vitamins. It is cheap, easily available, and can help in curing many diseases. The seeds of the plant have antidiabetic, antisnake venom, and hepatogenerative properties. Different parts of tamarind (fruit pulp, leaves, and stem bark) have antioxidant, analgesic, antiemetic, antibacterial, hepatoregenerative, and hypolipidemic activities. Apart from the other components of fruit, leaves, and seeds of tamarind are commercially and nutritionally valuable. The purpose of this review is to explore the phytochemical constituents, traditional uses, medicinal and pharmacologic activities of tamarind, and its use as a household preserve..

Keywords: *Tamarindus indica*; Antidiabetic activity; Pharmacological studies.

Introduction

Tamarindus indica belongs to the family Caesalpiniaceae, which is a sub-family in Leguminosae and has been widely cultivated since ancient times (between 1200 and 200 B.C.). It has been mentioned in *Caraka Samhita, Susruta Samhita,* and *Ashtanga Sangraha* with the name '*Amleeka*' under *Amla Varga* (group of sour drugs). It is cultivated mainly for the pulp in the fruit, which is used to prepare beverages and to flavor confections, curries, and sauces. The flower and leaf are eaten as vegetables [**1**]**.** The origin of tamarind is in central Soudan, West Africa. It is cultivated throughout almost the whole country, except in the Himalayas and western dry regions. Tamarind is used as a traditional medicine in India, Sudan, Nigeria, Bangladesh, and most of the tropical countries (from Africa to the Caribbean and South America and up to Southern Florida) [**2**]**.** Several carbohydrates, fats, proteins, tannins, essential amino acids, phytochemicals, vitamins, and minerals have been present in different parts. Tamarind has phenolic compounds such as catechin, epicatechin, procyanidin B_2, and other constituents like tartaric acid, mucilage, pectin, arabinose, xylose, galactose, glucose, uronic acid, and triterpenes. It possesses a large amount of vitamins B and C, which are responsible for the enhancement of the immune system [**3**]. In the West African sub-region, including Nigeria, it is widely used as both food and medicine. The pulp has antipyretic, antiscorbutic, laxative, carminative, digestive, expectorant, blood tonic, and remedy properties for biliousness and bile disorder, and the leaves have antihelmintic and vermifuge properties, destroying intestinal parasites [**4**]**.** Other parts of the plant have antidiabetic, antioxidant, antihepatotoxic, anti-inflammatory, antimutagenic, and many other activities [**5**]**.**

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Table 1: Vernacular Names [1, 6]

Language/ Country	Vernacular Names
Assamese	Tamar, Tenteli
Bengali	Tetula, Nuli, Tintil, Tinturi
English	Indian date, Tamarind tree
Gujarati	Amali, Ambali
Hindi	Amli, Imli
Kannada	Hunise,imli.
Marathi	Ambali, Amli, Chinch
Malayalam	Puli, valampuli, kolpuli.
Oriya	Tentuli
Punjabi	Imli
Sanskrit	Amlika, Chincha, Tinthidi, Chukra
Telugu	Chinta, Chintachettu, Chintapandu
Tamil	Amilam, Puli, Puliamavam
Arabic	Ardeib, Aradeib
Burmese	Magyee, Majee-pen
French	Tamarinde, Tamarinier
Indonesian	Asam, Asamjawa, Tambaring
Italian	Tamarindo
Nepali	Imli
Spanish	Tamarin, Tamarindo
Thai	BakhamMakham, Somkham
Vietnamese	Me, Trai me

Classification

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledoneae

Order: Fabales

Family: Fabaceae(Leguminosae)

Subfamily: Caesalpiniaceae

Genus: *Tamarindus*

Species: *T.indica*

Pharmacological Activities

Antidiabetic and Hypolipidemic Activity

Pulp and fruit extracts of tamarind show hypolipidemic and antioxidant activities in rats fed a cholesterol-rich diet **[5].** Ethanolic extract (50 mg/kg) of tamarind fruit pulp showed a significant decrease in body weight, serum cholesterol, triglycerides, and increased HDL cholesterol in cafeteria diet and sulpiride-induced obese rats [11]. Hyperglycemia, hyperlipidemia, and obesity are the main consequences of *diabetes mellitus*, metabolic syndrome, and cardiovascular problems. These metabolic abnormalities are controlled by tamarind [12]. Aqueous methanolic leaf extract showed significant protection and lowered the blood glucose level to normal. In alloxan-induced diabetic rats, the maximum reduction in glucose was observed after 6 hours at a dose level of 200 mg/kg of body weight. The significant antidiabetic activity of tamarind leaf may be due to inhibition of free radical generation and subsequent tissue damage induced by alloxan or potentiation of the plasma insulin effect by increasing pancreatic secretion of insulin from remaining beta cells [13]. Effect of tamarind seed extracts, recovered from subcritical water extraction (SWE), on testosterone production in male rats, under a high-fat diet, which leads to hypo-androgenic conditions. The authors reported that the tamarind seed extract prevented the harmful effects caused by a prolonged diet rich in fats, thus providing health benefits for endocrine function [14]**.**

Table 2: Phytochemical Composition.

Plant part	Compounds	References
Fruit Pulp	Amino acids (alanine, leucine, phenylalanine, proline, serine), different organic acids [tartaric acid (3-10%), acetic acid, citric acid, formic acid, malic acid, succinic acid],inverted sugar (25-30%), pectin,protein (87.9 gm/kg), and fat (19.1 gm/kg). Furan derivatives (44.4%), carboxylic acid (33.3%),Phlobatannine, grape acid, apple acid. **Essential oils:** Phenylpropanoids (safrole, cinnamic acid, ethyl cinnamate), methyl salicylate, some pyrazines, trans-2-hexenal, some alkyl thiazoles (2-ethylthiazole, 2-methylthiazole), and terpenes (limonene, geraniol).	**[7, 8]**
Leaves	Linonene, benzyl benzoate, invert sugar, pipecolic acid, citric acid, nicotinic acid, 1-malic acid, volatile oils [geranial, geraniol, limonene],pipecolic acid, lupanone, lupeol, orientin, isoorientin, vitamin B3, vitamin C, vitexin, isovitexin, benzyl benzoate, cinnamates, serine, beta-alanine, pectin, proline, phenylalanine, leucine, potassium, 1-malic acid, tannin, glycosidesperoxidize.	**[7]**
Pericarp	**Polyphenolics:**Proanthocyanidins (73.4%) in the form of catechin (2.0%), epicatechin (9.4%), procyanidin B2(8.2%), procyanidin (tetramers to hexamers) **Flavonoids:** Taxifolin,apigenin, eriodictyol, luteolin, naringenin	**[9]**
Rootbark	*n*-hexacosane, eicosanoic acid, β-sitosterol, pinitol, octacosanylferulate, 21-oxobehenic acid, apigenin, vitexin	**[10]**
Seeds	Monosaccharides (Arabinose, glucose), Polysaccharides [Cellulose, chitinase, galactose, mucilage, pectin, uronicacid, xylose], Campesterol, β-amyrin, β-sitosterol, lipids with fatty oils,total protein (15%) [palmitic acid, oleic acid, linoleic acid, eicosanoic acid, albuminoid. amyloids, phytohemaglutinins], and some keto acids. Oligomericprocyanidins, Bufadienolide (Scilliphraside 3-O-β-D glucopyranosyl (1-2)-L-rhamnopyranoside), and Cardenolide (Uzarigenin-3-O-β-D-xylopyranosyl (1-2)-α-L rhamnopyranoside)	**[7]**

Antioxidant activity

Hydro-alcoholic and aqueous extracts of tamarind leaf possess antioxidant activities like Fe^{+3}reducing potential, NO•, OH•, and DPPH• radical scavenging potential [**15**]. Caffeic acid is the most active compound (seed extract) for antioxidant activity and, therefore, capable of protecting cells against lipid peroxidation that has been identified in aging and many diseases such as cancer, cardiovascular disease, diabetes, and inflammatory diseases [**16**]. Tamarind seed coat also contains active antioxidants, phenolics, tannins, and flavonoids, and its extracts possess lipid peroxidation reduction, antityrosinase collagen stimulating, antimicrobial, anti-inflammatory, antidiabetic, and antihyperlipidemic activities **[17].** Pharmacological and toxicity effects of tamarind leaf extract in erythrocytes and their results showed that despite the presence of saponin, the extract worked as a protector of the cells, probably due to their antioxidant mechanisms and flavonoid content **[18, 19]**. Crude extract of tamarind pulp has phenolic compounds with antioxidant properties that have improved the efficiency of superoxide dismutase,

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catalase, and glutathione peroxidase in animals [**20**]. Flavonoids have been reported to exert multiple biological properties, but the best-described property of almost every group of flavonoids is their capacity to act as antioxidants, which can protect the human body from free radicals [**21**]**.** Oxidative stress is characterized by an imbalance in the generation of free radicals and their subsequent elimination by endogenous antioxidants. The phytochemical components and antioxidant potential of the ethyl acetate extract of tamarind leaves assess its capability to manage diseases associated with oxidative stress [22].

Hepatoprotective Activity

The hepatoprotective effect of tamarind was evaluated by intoxicating the rats with paracetamol (1gm/kg p.o.) for 7 days. The aqueous extracts of different parts of Tamarind such as fruits, leaves (350 mg/kg p.o.), and unroasted seeds (700 mg/kg p.o.) were administered for 9 days. The result showed that the tamarind extract caused a significant decrease in serum ALP (alkaline phosphatase), AST (aspartate aminotransferase), and bilirubin levels, significantly lowering liver weight and reducing necrosis [23]. Hydroalcoholic and aqueous extracts of tamarind seeds possess hepatoprotective and antioxidant activities. Significantly decreased the hepatic function test markers like SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase), ALP (alkaline phosphatase), and serum bilirubin, and significantly increased the antioxidant enzymes like GSH (glutathione), CAT (catalase), and SOD(superoxide dismutase), and significantly reduced lipid peroxidation [**24**]**.** Ethanolic extracts of tamarind flower showed hepatoprotective effects in Wistar rats when hepatotoxicity was induced by isoniazid and rifampicin [25]. In research, the effect of the trypsin inhibitor isolated from tamarind seeds (TTI), nano encapsulated in chitosan, and whey protein isolate (ECW) on the liver health status of the Wistar rats fed a high glycemic index (HGLI) diet were studied. In animals, ECW reduced ($p<0.05$) blood glucose (17%), glutamic oxalacetic transaminase (39%), and alkaline phosphatase (24%). Besides, ECW reduced ($p<0.05$) APRI (aspartate aminotransferase to platelet ratio index) and FIB-4 scores and presented a better aspect of hepatic morphology. ECW promoted benefits over a liver injury caused by the HGLI diet related to hyperglycemia and consequently, hepatic lipogenesis [26].

Anti-Inflammatory Activity

The perceived medicinal effects of tamarind leaves, bark, and pod husks relate to their anti-inflammatory activity and scarification or cicatrization (the formation of scar tissue) [**27**]. Leaf juice with ginger is used in the treatment of bronchitis, and the bark is dried, pounded, and added to water for the treatment of eye inflammation [**2**]**.**The leaves of this plant also showed inhibition of carrageenan-induced paw edema*via* membrane stabilization, neutrophils, and TNF-α synthesis inhibition [3]. In an additional in vivo study, the ethyl acetate seeds extract and the petroleum ether fraction of *Tamarindus indica* seeds significantly ($p<0.01$) increased latency to tail flick in the tail immersion method in Wistar rats and increased the mean basal reaction time in the hot plate method at particular doses of 50 mg/kg and 100 mg/kg body weight. Similarly, the methanolic seed extract of *Tamarindus indica* significantly ($p<0.01$) reduced paw edema stimulated by carrageenan in Wistar albino rats at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight [28]**.** The 95% ethanolic extract of *Tamarindus indica* seed was detected to be a potent reducer of pro-inflammatory mediators, arthritis-mediated cartilage, and bone degradation in adult Wistar rats at a dosage range of 25-50 mg/kg per day after 15 days of treatment [29].Trypsin inhibitor extracted from tamarind seeds (*Tamarindus indica* L.). Animals treated with trypsin inhibitor purified from tamarind (TTIp) also presented less TNF-α immunostaining in adipocytes and fewer plasma concentrations of this cytokine than non-treated animals [30].

Antimicrobial Activity

The methanolic leaf extract of tamarind was assessed for antibacterial activity against melioidosis, a life-threatening infection common among paddy cultivators in Southeast Asian countries. Antibacterial activity against *Burkholderia Pseudomallei* was exhibited by leaf extract in the disc diffusion test with a MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) value of 125µg/ml. Further animal studies for the role of tamarind are recommended by these *in vitro* inhibitory potentials for treating melioidosis [31]**.** Tamarind was considered for antimicrobial activity against some commongram-positive, gram-negative, and fungi. Plant extract activities were not affected when treated at different temperature ranges (4°C, 30°C, 60°C, and 100°C), but were reduced at alkaline pH. MIC and MBC of ethanolic extract of stem and leaf against bacteria were 15mg/ml(stem) and 18mg/ml (leaf) in *Escherichia coli*, 14mg/ml(stem) and 20mg/ml(leaf) in *Pseudomonas aeruginosa*, 10mg/ml(stem) and 15mg/ml(leaf) in *Salmonella typhi*, 20mg/ml(stem) and 20mg/ml (leaf) in *Staphylococcus aureus*, and 8mg/ml(stem) and 18mg/ml(leaf) in *Bacillus subtilis.* The result shows that *Staphylococcus aureus* had a high MIC and MBC (20 mg/ml), and *Bacillus subtilis* had the lowest MIC and MBC (8mg/ml). Thus, plant extracts are effective against gram-negative and gram-positive bacteria [4,32].

Anthelmintic Activity

Leaf and bark extracts of tamarind have anti-helminthic activity. Alcoholic extract of the bark of tamarind caused paralysis at 22.33 minutes and times of death at 45.00 minutes for *Pheritima posthuma*, and 14.66 minutes as paralysis time, and 20.66 minutes as times of death for *Tubifex tubifex* worms, respectively. The aqueous fraction treatment of *Pheritima posthuma* and *Tubifex tubifex* worms resulted in a paralysis time of 58.33 and 23.00 minutes, respectively [33]. Tamarind leaf juice has an anthelmintic effect against *Pheretima posthuma*, as a test worm. Various concentrations (100%, 50%, and 20%) of tamarind leaf juice were tested in the assay, which involved the determination of paralysis (P) and death (D) of worms. It shows a shorter time of paralysis (P=23.5 min) and death (D=62 min) in 100% concentration, while the time of paralysis and death will increase in 50% concentration (P=26 min and D = 65 min.) and 20% concentration (P=30 min.and D=72 min.), respectively, as compared to piperazine citrate (10mg/ml) used as a standard drug (P=23 min. and D=60 min.) [34]**.**

Acaricidal Activity

The crude extract of tamarind with water and 10% ethanol

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in water was tested for acaricidal activity on the engorged female cattle tick (*Boophilus microplus)* by the dipping method. The mature tamarind fruit extract was obtained by taking off the seeds with water or 10% ethanol in a ratio of 1:2 and 1:5 W/V for 7 days. The corrected mortality of the ticks was observed after dipping at 24 hours, 48 hours, and 7 days. The corrected mortality means of ticks in crude extracts of tamarind fruits were 56-70%, 70-89%, and 77-99%, with no statistically significant difference after dipping at 24 hours, 48 hours, and 7 days, respectively. By the dipping method, acaricidal activity was also bioassayed by organic acids in tamarind fruits (oxalic, malic, succinic, citric, and tartaric acids). 0.5% and 1% oxalic acid concentrations showed the highest acute acaricidal activity (56% and 62% mortality of ticks at 24 h after dipping, respectively). 1% tartaric acid concentration showed the highest delayed acaricidal activity (73% mortality of ticks at 7 days after dipping). A 0.5%mixture of oxalic acid with 0.5% malic, succinic, citric, and tartaric acid concentrations of 1:1 V/V was tested for acaricidal activity. The result showed that the acaricidal activity of these acid mixes was not more durable than that of each single acid **[32].**

Analgesic Activity

Various extracts of tamarind bark were screened for analgesic activity by using suitable models such as a hot plate test and an acetic acid-induced writhing test. The petroleum ether extract showed significant results at 50 mg/kg, i.p., as compared to the standard drug pentazocine (10 mg/kg, i.p.). Some sterols and triterpenes are responsible for anti-inflammatory and analgesic activity [**7**]. The aqueous fruit extract (60-600mg/kg) significantly inhibited the writhing test in a dose-independent manner, with the percentage of analgesia recorded between 51.8 and 74.1%. In addition, the extract also significantly increased the latency time in the hot plate test in a dose-dependent manner and also showed inhibitory activity in both the early and late phases of the formalin test. Besides, pre-treatment with 5mg/kg of naloxone, a non-selective opioid receptor antagonist, significantly modified the antinociceptive effect in all tests. At both the peripheral and central levels, aqueous tamarind fruit extract has potential antinociceptive activity, which is mediated *via* activation of the opioidergic mechanism **[35].**

Wound Healing Activity

The ability of the polysaccharide from the tamarind seed (xyloglucans) to repair corneal wound healing might depend on its influence on the integrin recognition system (*in vitro*, with cultured human conjunctival cells) [**36**]. Tamarind is frequently cited in the literature regarding the treatment of cuts, wounds, and abscesses. Tamarind bark or leaves are most commonly used on the spot, either externally, alone or in combination with other species [**37**]. Other tamarind plant parts are also used in wound healing medicine, such as fruit, leaf powder, pod husks, or gum [**38**]. A decoction of tamarind leaves was one of the most important agents for cleaning wounds caused by Guinea worm infections. A decoction of the leaves may be used to wash wounds, ulcers, lesions, or sores in the mouth [**27**]. Tamarind seeds showed significant wound healing activity on epidermal circular wounds, with enhancement of wound closure and antioxidant function [**39**].

The Anti-Snake Venom Activity

Tamarind seed extract evaluated the pharmacological and enzymatic effects induced by *Viper russelli* venom. Hyaluronidase, protease, PLA (2), l-amino acid oxidase, and 5'-nucleotidase enzyme activities of venom were inhibited by tamarind seed extract in a dose-dependent manner. These are the major hydrolytic enzymes responsible for the early effects of envenomation, such as local tissue damage, inflammation, and hypotension. The seed extract neutralized the degradation of the beta-chain of human fibrinogen and indirect hemolysis caused by venom. The extract prolonged the clotting time moderately. Venom-induced edema, hemorrhage, and myotoxic effects were reduced significantly when different doses of seed extract were pre-incubated with venom before the tests. After 10 minutes of biting (injection of venom) animals that received seed extract were safe from venom-induced toxicity. Since it inhibits hydrolytic enzymes and has pharmacological effects, it may be used as an alternative treatment to serum therapy and, in addition, as a rich source of potential inhibitors of PLA(2), metalloproteinases, serine proteases, hyaluronidases, and 5-nucleotidases, the enzymes involved in several physiopathological human and animal diseases [**40**].

Anti-diarrheal and Anti-dysentery Activity

In tropical countries, diarrhea is one of the major health problems and often occurs during rainy weather. There appears to be a rare difference between West and East Africa in the cure of diarrhea. For West Africa, literature only mentions the use of the bark. It can be applied as a decoction, pulped with lemon, or macerated in milk. In East Africa, it is not the bark but the leaf that is used, made into a juice or beverage, or prepared in a concoction with *Sterculia Africana*. In Kenya, the use of ground seeds has been recorded, and in Tanzania, the root is used to treat dysentery [**27**]. Tamarind pulp with lemon is used to treat diarrhea (anti-diarrheal activity), and the root is used to treat dysentery (anti-dysentery activity). Dysentery is a type of diarrhea containing mucus or blood, usually caused by an infection of the intestine. When diarrhea is not treated properly, the patient has a risk of dehydration and death [**41**].

Abdominal pain

Abdominal pain is not a specific disease but a complaint that mentions a painful abdomen and may have a wide variety of causes, including constipation or diarrhea. Soaked fruits are also eaten by rural Fulani in Nigeria to get rid of constipation. Roots prepared as an extract are used in the cure of stomach aches or painful abdomen, mostly in East Africa [**42**]. Root extract is used in the treatment of stomach aches or painful abdomen, mainly in East Africa and also in Burkina Faso. It is used for abdominal pain and related complaints. The pulp of tamarind pods is so gentle that it laxatively increases the common slowness of the bowels. Due to the presence of a high amount of malic acid, potassium acid, and tartaric acid, the fruit is usually used as a laxative. Whole tamarind fruits are given to children in Madagascar for breakfast to control constipation. Bengal (a sweetmeat) formed from the unripe fruit of tamarind is used as a laxative as such or sometimes mixed with lime juice or honey by people in Senegal [**27**].

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Anti-atherosclerosis

Effects of crude extract of tamarind pulp fruit on lipid serum level and early atherosclerotic lesions in hypercholesterolemic hamsters *in vivo*, and antioxidant action *in vitro*. Tamarind 5% fruit pulp extract led to a reduction in serum cholesterol (50%), non-HDL cholesterol (73%), and triglycerides (60%) and an elevated HDL-cholesterol level (61%) in hypercholesterolemic hamsters. In *in-vitro*, the extract showed radical scavenging ability as assessed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radicals'tests and reduced lipid peroxidation in serum as measured by the thiobarbituric acid reactive substances (TBARS). In *in-vivo*, the extract also improved the efficiency of the antioxidant defense system as assessed by superoxide dismutase, catalase, and glutathione peroxidase activities. Together, these results indicate the potential of tamarind (pulp fruit) extracts to diminish the risk of atherosclerosis development in humans **[5].**

Fever and malaria

Fruits are known as a febrifuge in Madagascar and throughout the Soudan. In Benin and Sudan, the fruits were used to treat malaria. Malaria was treated with tamarind leaves in Ghana, Benin, and Nigeria. Fruit pulp was used as a febrifuge and a laxative in the Sahel and Soudan regions. Both problems were not only treated with the same ingredient all across the savannah belt from Senegal to Ethiopia, but records of identical recipes based on tamarind fruit pulp exist for the treatment of malaria, fever, and constipation. That is the case in Senegal, Benin, and Sudan, where the recipe involves preparing a solution of tamarind pulp and water, sometimes involving a boiling step **[27]**. In a study of antimalarial activity of the tamarind pulp extracts against *Plasmodium falciparum*, they were extracted with solvents of different polarities. Among the solvents used, the chloroform solvent showed the highest activity, as it contained mainly aliphatic hydrocarbons, acid alcohols, and their esters, in addition to sitosterol and aromatics. This study indicated that the antispasmodic activity is due to one or a group of these components **[43]**.

Effect on Cardiovascular System and Blood

The effect of pulp crude extract in hypercholesterolemic hamsters was studied on lipid serum levels and atherosclerotic lesions. Tamarind extract has a high potential for reducing the risk of atherosclerosis in humans **[5]**. In Bangladesh, tamarind fruits were evaluated for their effects on the lipid profile, systolic and diastolic blood pressure, and the body weight of humans. Another experimental study on hamsters has shown that the hydroalcoholic extract of tamarind pulp influences the mediator system of inflammation **[41].**

Conclusion

This review gives broad information about the bioactive constituents, ethnopharmacology, and medicinal uses. Tamarind, which includes a variety of bioactive compounds in the leaves, seeds, bark, fruit pulp, and flowers, possesses a large range of nutritional properties with beneficial effects on human health and the possibility of application in the pharmaceutical and textile industries. The sweet and sour taste at the same time in the fruit is unique due to its ingredients, and it is used popularly in cooking. It is often more difficult to determine which use is more important: food and beverages or natural medicine. This proves the therapeutic importance of the plant. This review is a simple step taken to compile the classical literature on tamarind and hopefully induce advanced research about the benefits of this plant for human life.

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Conflict of interest

The authors declare that they do not have any conflicts of interest.

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