

## Phytochemistry and medicinal potential of the *Terminalia bellirica* Roxb. (*Bahera*)

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*Terminalia bellirica* Roxb., known as *Bahera* or *Beleric* or *bastard myrobalan*, belonging to the family *Combretaceae* of order *Rosales*, is a large deciduous tree common on plains and lower hills in Southeast Asia, where it is also grown as an avenue tree. Glucoside, tannins, gallic acid, ellagic acid, ethyl galate, gallyl glucose, chebulanic acid are the main active phytoconstituents of medicinal importance. These phytoconstituents are responsible for many of the pharmacological roles. Different parts of the tree have various medicinal activities viz., antisecretory, analgesic, antihypertensive, antidiarrhoeal activity, antimicrobial activity, antidiabetic, antioxidant, antiulcer, antipyretic, hepatoprotective, anticancer, angiogenesis, antidepressant-like and anti-urolithiatic activity. This affects the LDL oxidation and macrophage inflammatory response and also nephrotoxic effects. Also having antipsychotic potential helpful in preventing delaying clot formation and have immunostimulant activity. Their traditional uses include relief in a cough, asthma, indigestion, dental problems, sore throat and wounds. The review also records some tissue culture investigations made for micropropagation and genetic diversity of *T. bellirica*.

**Keywords:** Dental problems, Folk medicine, *Terminalia bellirica* Roxb., Phytopharmacology, Phytoconstituents.

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### Introduction

Medicinal plants have been used in all cultures as a source of medicine since times immemorial. Herbal medicine is still the mainstay of health care in several developing countries. The World Health Organization has estimated that more than 80 % of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs<sup>1</sup>. *Terminalia bellirica* Roxb. is a large tree, up to 40 m high. Leaves are petiolate, broadly elliptic, clustered towards the end of branches. Flowers are greenish yellow, in solitary, simple, axillary spikes. *T. bellirica* is found in deciduous forests throughout the greater part of India, but not in the arid regions, in upper Gangetic plains, Chota Nagpur, Bihar, Orissa, West Bengal, Konkan, Deccan and most of South India<sup>2,3</sup>.

The common name *Bahera* or *Bhaira* is based on the Hindi name of the tree. The word *Bellirica* is taken from the scientific name of the tree and distinguishes this myrobalan from the other one (*Chebulic myrobalan*)<sup>4</sup>. It is the secondary host of tasar silkworm<sup>5</sup>. *T. bellirica* is known as *Vibhitaka*, *Vibhitaki* in Sanskrit, *Belliric myrobalan* in English, in Tamil-*Tanni*, *Tanrikkai*, in Punjabi- *Bahera*, *Birha*.

*T. bellirica* contains different chemical constituents in different parts such as stem bark contains arjungenin

and its glycosides, belleric acid, bellericosides. Fruits contain *hexahydroxydiphenic* acid, methyl ester,  $\beta$ -sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose, and rhamnose<sup>6</sup>. *T. bellirica* bark is mildly diuretic and useful in anaemia and leucoderma. Fruits are anti-inflammatory, antihelmintic, expectorant, antipyretic, antiemetic and useful in asthma and bronchitis, dropsy, dyspepsia, cardiac disorders, skin diseases, leprosy, ulcer. Ripe fruits are used as astringent in combination with chebulic myrobalan (*Terminalia chebula*) and *Phyllanthus emblica* as the famous Triphala drug of Ayurveda are also useful in eye problems like cataract, glaucoma, progressive myopia, and conjunctivitis<sup>7</sup>. The bark of *T. bellirica* is used as an adulterant to the bark of *T. arjuna*<sup>8</sup>. The deciduous tree *T. bellirica* found in Southeast Asia is extensively used in traditional Indian Ayurvedic medicine for the treatment of hypertension, rheumatism and diabetes<sup>9</sup>.

Glucosides, tannins, galliic acid, ellagic acid, ethyl gallate, gallylglucose, chebulanic acid are believed to be mainly responsible for its wide therapeutic actions. It has anti-HIV-1, antimalarial and antifungal activity<sup>10</sup>. It is used as antioxidant, antimicrobial, antidiarrheal, anticancer, antidiabetic, antihypertensive and hepatoprotective agent<sup>11</sup>. It also possesses analgesic, antipyretic and anti-ulcerogenic effect and antimicrobial activity<sup>12-15</sup>.

Research investigations on *T. bellirica* explored its phytochemical and medicinal attributes, but the data

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available is quite scattered. This review is to systematically record the literature available to date on phytochemistry, ethnopharmacology, toxicology, pharmacological profile and also the tissue cultural investigations.

### Ecology

*T. bellirica* is a large deciduous tree found throughout India, in areas up to an altitude of 1,000 m. The tree attains a height of 30-40 m. The alternate, broadly elliptic leaves are clustered towards the end of the branches. They are 10 to 12 cm in length and 7-14 cm in breadth. The tree is found in abundance in Madhya

Pradesh, Uttar Pradesh, Punjab and Maharashtra. It is known as *vibhitaki*, *karshaphala* and *kalidruma* in Sanskrit. Flowers: small, dirty-grey or greenish-yellow with a strong offensive smell, found in axillary spikes. Fruits: ovoid, grey, velvety, drupes contain single hard stony seed and obscurely 5-angled when dry. Flowers start in March-May and fruits ripen in December-February. The bark is brownish grey in colour.

### Phytoconstituents

Time-to-time researchers have investigated *T. bellirica* for its many biologically active phytochemicals. They are presented in Table 1.

Table 1 — Phytochemical constituents reported from the Baheda –*T. bellirica*

Source	Phytochemicals	References
Plant body	Beta cetosterol, tannins, gaelic, ethyl and ellagic acid	16
	Gallo-tannic acid, Coloring matter, resins and a greenish yellow oil, Tannins, ellagic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyllembin, $\beta$ -sitosterol, mannitol, glucose, fructose and rhamnose	17
	Glucoside (bellericanin), Gallo-tannic acid	18
	Ellagic acid, gallic acid, lignans (termilignan and thannilignan), 7-hydroxy 3'4' (methylenedioxy) flavone and anolignanB	19
	Tannins, ellagic acid, ethyl gallate, galloyl glucose and chebulagic acid, phyllembin, $\beta$ -sitosterol, mannitol, glucose, fructose and rhamnose	20
Seed	Cardenolide, cannogenol 3-O-D-galactopyranosyl14-O-L-rhamnopyranoside and phospholipids 14.	13
	Seed contained 12.28 % oil on dry basis; Moisture, ash and crude fibre contents of the seed kernel were found to be 8.43, 2.54, and 8.78 % respectively. The refractive index, co-efficient of viscosity, specific gravity, and energy of activation of the oil were found to be 1.28, 403.6 millipoise at 30Å °C, 0.93 and 6.97 Kcal/mole respectively; Iodine value, acid value, peroxide value, saponification value, saponification equivalent, ester value, unsaponifiable matter, acetyl value, Reichert-Meissel value, Polenske value, free fatty acids as oleic acid and cholesterol content of the oil 107, 3.69, 3.14, 189.24, 296.44, 185.55, 1.24 %, 3.78, 0.719, 0.945, 0.87 % and 26.59 mg per 100 g oil, respectively. ;17.70 % myristic acid, 21.6 % palmitic acid, 45.67 % oleic acid and 14.93 % stearic acid.	21
Fruit	Tain termilignan, thannilignan, 7-hydroxy-3',4'-(methylenedioxy) flavones, anolignan B 5, gallic acid, ellagic acid, $\beta$ -sitosterol	22, 23
	Two new lignans named termilignan and thannilignan, together with 7-hydroxy-3',4'-(methylenedioxy) flavan and anolignan B	10
	Flavonoids, sterols and tannins	14
	2, 3, 7, 8 -Tetrao xy-chro meno (5,4,3-cde) chro mene-5,10-dione,	24
	Alkaloid, antraquinone glycoside, saponins, flavonoids, polysaccharides, Steroid, Tannin	8
	Arjungenin, belleric acid, bellericoside, cannogenol 3-O-dgalactopyranosyl-(1g4)-O-l-rhamnopyranoside sitosterol, ethyl gallate, chebulagic acid, galloyl glucose, mannitol, glucose, galactose, fructose and rhamnose	6, 25
	Gallic acid, ellagic acid and chebulagic acid	26
	Gallo-tannic acid and glycoside bellericanin	27
	Alkaloid, phenol, tannins and flavonoids.	28
	Glycosides, flavonoids, tannins, phenols, saponin, carbohydrates and proteins.	29
Stem bark	Arjungenin and its glycosides, belleric acid and bellericosides.	13
	Hydrolysable tannins; gallic acid and ellagic acid in the water-soluble extract	30
Leaf	Proteins, steroids and terpinoids	31
	Three hydrolysable tannins; gallic acid , ellagic acid and methyl gallate; one flavone; luteolin; two flavonol aglycones; quercetine and kaempferol; and four flavonol glycosides; quercetin 3-O-[6"- a-L-rhamnopyranosyl]-(1g 6)- $\beta$ -D- glucopyranoside (rutin.), quercetin-3-O-a-L-rhamnopyranoside,quercetin3-O- $\beta$ -Dglucopyranoside and kaempferol 3-O- $\beta$ -D-glucopyranoside	32
	Saponins, Tannins, Amino acids, Proteins, Alkaloids, Carbohydrates and Flavonoids.	33
	4-hydroxy-(2-methylbutanol) benzoic acid	34
	Pyridine-3-carboxamide,4-dimethylamino-N,2,4-difluorophenyl $\beta$ -sitosterol,1,5-diphenyl-3-pentanone, 9-Octadecenoic acid	35

**Medicinal activities**

In the current era, herbal products are considered to be the symbols of safety in comparison to the synthetic products that are regarded to be hazardous to human life and the environment. So far known medicinal potential of *T. bellirica* is compiled in Table 2.

**Anticancer activity**

The studies on the anticancer properties of *T. bellirica* have proved to be a useful asset for preparing a potential anticancer drug. In an *in vitro* experiment carried out to know about the anticancer activity of 70 % methanolic extract of *T. bellirica* (TBME) against human breast (MCF-7) carcinoma and human lung (A549) carcinoma

Table 2 — Medicinal Potential of Baheda – *T. Bellirica*

Source	Observed medicinal potential	References
Plant body	Antisecretory and analgesic activities-crude extract inhibited the castor oil-induced intestinal fluid secretion in mice at the dose range of 300 - 1000 mg/kg and extract also dose-dependently (50 - 100 mg/kg) reduced the numbers of acetic acid-mediated writhes in mice	36
Fruit pulp	Antidiarrhoeal activity-Aqueous and ethanolic extract of fruit pulp at the doses of 334 mg/kg, 200 mg/kg, 143 mg/kg showed antidiarrhoeal activity	37
Plant body	Antihypertensive effect-crude extract of <i>T. bellirica</i> fruit (Tb.Cr) induced a dose-dependent (10-100 mg/kg) fall in the arterial BP of rats under anaesthesia. In isolated guinea-pig atria, Tb.Cr inhibited the force and rate of atrial contractions. In the rabbit thoracic aorta, Tb.Cr relaxed the phenylephrine (PE, 1 $\mu$ M) and K <sup>+</sup> (80 mM)-induced contractions as well as suppressed the PE (1 $\mu$ M) control peaks in the Ca <sup>++</sup> -free medium, similar to that caused by verapamil. It lowers BP through Ca <sup>++</sup> antagonist mechanism and thus provides a sound mechanistic background for its medicinal use in hypertension	14
Fruits	Alcoholic and aqueous extracts of TB showed significant anti salmonella activity	38
Fruits	Elicited relaxation of spontaneous contractions in both isolated rabbit jejunum and guinea-pig ileum, showed a protective effect against castor oil-induced diarrhoea and carbachol-mediated bronchoconstriction in rodents, in the guinea-pig trachea, relaxed the CCh-induced contractions	39
Dry fruits	Antimicrobial activity-found activity in an aqueous extract of dry fruit against 9 human microbial pathogens @ 4 mg concentration showed the highest zone of inhibition against <i>S. aureus</i> and dry fruit powder possessed potential broad-spectrum antimicrobial activity	40
Plant body	Ethanolic extract of the plant showed strong activity against <i>Streptococcus mutans</i>	41
Leaf extract	Promising antibacterial activity especially towards Gram-negative bacteria. The inhibitory zone of <i>Escherichia coli</i> is 16.3 $\pm$ 1.02, 19.0 $\pm$ 0.12, 22.6 $\pm$ 1.08 in <i>Pseudomonas aureus</i> inhibition of zone is 12.2 $\pm$ 0.14, 16.0 $\pm$ 1.04, 23.4 $\pm$ 0.06 and for <i>Streptococcus pyrogens</i> , the inhibition is 18.3 $\pm$ 1.04, 21.3 $\pm$ 0.08, 24.0 $\pm$ 1.02 respectively and found the zone of inhibition of <i>Streptococcus pyrogens</i> with 400 $\mu$ g/mL of <i>T. bellirica</i> equal to that of standard	33
	The aqueous extract exhibited significant activity against the tested bacteria- <i>E. coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumonia</i> , <i>Shigella flexneri</i> , and <i>Salmonella typhi</i> and fungal isolates- <i>Aspergillus niger</i> , <i>Mucor</i> species, <i>Aspergillus fumigatus</i> , <i>Rhizopus</i> species and <i>Aspergillus flavus</i> , compared with chloroform and petroleum ether extract respectively	28
	70 % methanol, ethyl acetate, butanol and aqueous extracts showed good antibacterial activity against <i>Micrococcus flavus</i> , <i>Staphylococcus aureus</i> and <i>E. coli</i> compared to the antibiotics streptomycin and ampicillin	32
Leaf and stem powder	good against Gram-positive bacteria like <i>Corynebacterium rubrum</i> and <i>Staphylococcus epidermidis</i> and Gram-negative bacteria like <i>Klebsiella pneumonia</i> , <i>E. coli</i> and <i>Salmonella typhimurium</i> .	42
Leaf	Herbal paste preparation showed significant ( $p < 0.05$ ) improvement in maturation, wound contraction and epithelialization, offers a distinctive advantage in wound healing	43
	Immunological activity-extract affected T cell proliferation, also affected B cell proliferation through T cell-independent and T cell-dependent mechanisms respectively. Also found that the extract affected cellular mediated immunity (CMI) rather than humoral mediated immunity (HMI)	19
Fruits	Antidiabetic activity-Hexane, ethylacetate and methanolic extracts of <i>T. bellirica</i> fruit @ 200, 300 and 400 mg/kg, p.o for 60 days to Streptozotocin-induced diabetic rats significantly ( $p < 0.05$ ) increased the plasma insulin, C-peptide and glucose tolerance levels, body weight, serum total protein, in addition significantly decreased the serum levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, urea, uric acid and creatinine in diabetic rats	44
	Glucose utilization in <i>Vero</i> , L6 and 3T3 cell lines showed that the ethanolic extract was found to be prominent, <i>Vero</i> , L6 and 3T3 cell lines enhance the glucose uptake by 27.15 $\pm$ 1.19, 18.73 $\pm$ 1.29 and 24.43 $\pm$ 0.88 at 500 $\mu$ g/mL concentration	45

(Contd.)

Table 2 — Medicinal Potential of Baheda – *T. Bellirica*—(Contd.)

Source	Observed medicinal potential	References
	Administration of <i>T. bellirica</i> fruits against alloxan-induced hyperglycemia and antioxidant defence mechanism in rats showed a significant reduction of glucose level and oxidative stress was observed. Increased levels of antioxidant enzymes such as superoxide dismutase, glutathione reductase and catalase were observed in blood and liver	46
	The antioxidant activity-Antioxidant potential of acetone extract/fractions of its fruit was investigated using <i>in vitro</i> assays, including scavenging ability against 2,2'-diphenyl-2-picrylhydrazyl (DPPH), $\beta$ -carotene bleaching inhibition, reducing power and chelating ability on $Fe^{2+}$ ions. Maximum antioxidant activities (expressed as $EC_{50}$ values) observed were 14.56, 27.81 and 67.8 $\mu$ g/mL in DPPH, $\beta$ -carotene bleaching and reducing power assays, respectively	47
	Crude methanolic extract along with its various organic fractions elicited both <i>in vitro</i> and <i>in vivo</i> antioxidant activity as well as antibacterial activity.	48
	Antioxidant activity using the DPPH assay indicate that methanolic extracts from <i>Terminalia</i> possess significant antioxidant activity at concentrations of 1000-100 $\mu$ g/mL compared to control	35
	Antiulcer activity –Ethanol extract of <i>T. bellirica</i> at doses of 250,500 mg/kg orally produced significant inhibition of the gastric lesions induced by pylorus ligation induced ulcer and ethanol-induced a gastric ulcer. The extract (250 mg/kg & 500 mg/kg) showed significant ( $p < 0.05$ ) reduction in free acidity and ulcer index as compared to control	49
	Methanolic extract of fruits (100, 250, 500, 1000 mg/kg, p.o.) significantly suppressed the peptic ulcer induced by ethanol. Methanolic extract at a dose of 500 mg/kg showed, promising antiulcer activity hence, this dose was selected for further evaluation of antiulcer studies. The methanolic extract (500 mg/kg) showed significant ( $p < 0.05$ ) reduction in gastric volume, free acidity, total acidity, ulcer index, protein and pepsin content and increase in mucus content in pylorus-ligated rats as compared to control	50
	Delay in clot formation- fruit extract @ 0.20 mg/dL concentration showed maximum delay (more than 90 minutes) in clot formation	14
	Antipyretic activity-ethanolic and aqueous extracts of fruits (200 mg/kg, p.o.) in brewer's yeast-induced fever in mice and rats and showed a significant inhibition of elevated body temperature when compared to a corresponding control	51
Fruits	Hepatoprotective activity- evaluated the protective effect of fruit extract and its active principle, gallic acid (3,4,5-trihydroxybenzoic acid) at different doses against carbon tetrachloride intoxication. Toxicant caused a significant increase in the activities of serum transaminases and serum alkaline phosphatase. Hepatic lipid peroxidation level increased significantly whereas significant depletion was observed in reduced glutathione level after carbon tetrachloride administration. A minimum elevation was found in protein content, on the contrary, a significant fall was observed in the glycogen content of liver and kidney after toxicant exposure. Activities of adenosine triphosphatase and succinic dehydrogenase inhibited significantly in both the organs after toxicity. Treatment with extract (200, 400 and 800 mg/kg, p.o.) and gallic acid (50, 100 and 200 mg/kg, p.o.) showed dose-dependent recovery but the effect was more pronounced with gallic acid and found to be most effective against carbon tetrachloride-induced liver and kidney damage. Treatment with extract (200, 400 and 800 mg/kg, p.o.) and gallic acid (50, 100 and 200 mg/kg, p.o.) showed dose-dependent recovery in biochemical parameters such as SGOT, SGPT and lipid peroxidase, glutathione but the effect was more pronounced with gallic acid	52
Fruit powder	Antipsychotic potential-administration of <i>T. bellirica</i> fruit powder in a specially prepared diet for 15 successive days in different concentrations (4, 6 and 8 % w/w) showed significant ( $p < 0.05$ , $p < 0.01$ ) dose-dependent potentiating of haloperidol-induced catalepsy. Thus, the results suggest an antidopaminergic activity	53

and its possible mechanism, the extract showed significant cytotoxicity to both A549 and MCF-7 cells, while, in non-malignant WI-38 cells, no cytotoxicity was found. Flow cytometric analysis of A549 and MCF-7 carried out by taking 100  $\mu$ g/mL of TBME as the effective concentration inducing apoptosis in the cancer cell lines. This concentration of TBME proved to cause DNA fragmentation pattern of apoptosis. To know the mechanism of apoptosis induction, Nishida *et al.*<sup>54</sup> performed western blotting in which the ratio of Bax/Bcl-2 in both A549 and MCF-7 had increased, which in turn activated the caspase cascade and the cleavage of PARP.

These results confirmed the anticancer effects of TBME in both lung and breast cancer cell lines by modifying the Bcl-2 family proteins<sup>54</sup>.

Growth inhibitory activities and synergistic effects of *P. emblica* extract/ doxorubicin or cisplatin and *T. bellirica* extract/ doxorubicin or cisplatin extracts showed certain selective effects against A549 and HepG2 cells (two cancer cell lines)<sup>55</sup>. In another study, the antimutagenic effect of two polyphenolic fractions (TB-3, TB-4) isolated from *T. bellirica* in two strains of *S. typhimurium* (i.e. TA98 and TA100) against NPD (4-nitro-o-phenylenediamine), 2AF

(2-aminofluorene) and 4NQNO (4-nitroquinoline-N-oxide) have been distinguished. The researchers found that both the compounds were considerably effective against S9-dependent 2AF; rather having less striking effect against 4-O-nitrophenylenediamine (NPD) but almost no effect against 4NQNO in TA100 strain<sup>56</sup>.

Antimutagenic potency was observed in water, acetone, and chloroform extracts of *T. bellirica* through Ames Salmonella/microsome assay. Acetone extract exhibited the variable inhibitory activity of 65.6 and 69.7 % with 4-O-nitrophenylenediamine (NPD) and sodium azide, respectively (as direct-acting mutagens) and 81.4 % with 2-aminofluorene (2AF) (an S9-dependent mutagen), in the preincubation mode of experimentation. Inhibition with chloroform and water extracts was rather insignificant<sup>57</sup>.

The leaf extracts of *T. bellirica* have promising anticancer activity with IC<sub>50</sub> of 5.65 µg/mL. Phytochemical studies have revealed that *T. bellirica* contains a variety of bioactive ingredients with reported therapeutic activities. Methanolic *terminalia* leaf extracts (MTLE) demonstrated a dose-dependent inhibitory activity with a certain degree of selectivity against cell line Hep G2 cell line. MTLE supplementation down regulated expression of Bcl-2, a potent suppressor of apoptosis and induced expression of proapoptotic proteins Bax, caspase-9 and caspase-3 in a dose-dependent manner. It also demonstrated that the proapoptotic effect of MTLE was due to down regulation of the Akt/mTOR signalling pathway. Flow cytometry analysis showed an increase in the percentage of G2/M arrest phase in Hep G2 cell line compared to control cell line. In summary, the findings demonstrate that MTLE treatment of the Hep G2 cells resulted in significant (a) cell growth inhibition, (b) G2/M-phase cell cycle arrest, and (c) apoptosis in a dose-dependent fashion by triggering caspase-9 and caspase-3 activation. Overall, these findings suggest that *T. bellirica* leaves can serve as potential therapeutic for the cancer<sup>35</sup>.

#### Angiogenesis activity

Angiogenesis represents an excellent therapeutic target for the treatment of cardiovascular diseases. It is a potent physiological process that underlies the natural manner in which our bodies respond to a diminution of blood supply to vital organs, namely the production of new collateral vessels to overcome the ischemic state.

Prabhu *et al.*<sup>31</sup> evaluated the angiogenic potential of *T. bellirica* by *in vivo* mice sponge implantation assay. Here, gelatin sponge with or without ethanolic extract

of *T. bellirica* leaf (EETB - 0.3 and 0.5 mg, respectively) was subcutaneously injected into Swiss albino mice and after 14 days the implanted sponges were excised and stained section showed that sponge containing EETB had produced more vessels in gels than sponges alone. The new vessels were abundantly filled with intact Red blood corpuscles (RBCs), which indicate the formation of a functional vasculature inside the sponges and blood circulation in newly formed vessels by angiogenesis induced by EETB. Haemoglobin in control was nearly 0.3 µg, EETB cases the haemoglobin quantity was markedly enhanced to about 17 µg. Thus ethanolic extract of *T. bellirica* leaf exhibited a profound angiogenic activity *in vivo*.

#### Antidepressant activity

Dhingra and Valecha<sup>58</sup> investigated the effect of aqueous and ethanolic extracts of *T. bellirica* on depression in mice using forced swim test (FST) and tail suspension test (TST). The extracts were administered orally for 10 successive days in separate groups of Swiss young male albino mice. Aqueous extract (50, 100, and 200 mg/kg) in a dose-dependent manner and ethanolic extract (100 mg/kg) significantly reduced the immobility time of mice in both FST and TST. The extracts had no significant effect on the locomotor activity of mice. The efficacies of aqueous extract (200 mg/kg) and ethanolic extract (100 mg/kg) were found to be similar to that of imipramine (15 mg/kg, po) and fluoxetine (20 mg/kg, po) administered for 10 successive days. Both extracts reversed reserpine-induced extension of immobility period of mice in FST and TST. Prazosin (62.5 µg/kg, ip; an alpha1-adrenoceptor antagonist), sulpiride (50 mg/kg, ip; a selective D2 receptor antagonist) and p-chlorophenylalanine (100 mg/kg, ip; an inhibitor of serotonin synthesis) significantly attenuated the aqueous and ethanolic extract-induced antidepressant-like effect in TST. Thus, both the aqueous and ethanolic extracts of *T. bellirica* elicited a significant antidepressant-like effect in mice by interaction with adrenergic, dopaminergic and serotonergic systems.

#### Effects on LDL oxidation and macrophage inflammation

Tanaka *et al.*<sup>9</sup> investigated the effect of *T. bellirica* extract (TBE) on low-density lipoprotein (LDL) oxidation and inflammation in macrophages. TBE showed 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and 15-lipoxygenase inhibitory activity. TBE also significantly inhibited free radical-induced LDL oxidation compared to the solvent control

*in vitro*. In THP-1 macrophages, TBE treatment resulted in significant decreases of the mRNA expression of tumour necrosis factor-alpha (TNF), interleukin-1beta (IL-1), and lectin-like oxidized LDL receptor-1 (LOX-1). TBE also reduced matrix metalloproteinase (MMP)-9 secretion and intracellular reactive oxygen species (ROS) production in THP-1 macrophages. These results show that TBE has the inhibitory effects on LDL oxidation and macrophage inflammatory response *in vitro*. This suggests that its *in vivo* use may inhibit atherosclerosis plaque progression.

#### Anti urolithiatic effect

Upadhyay *et al.*<sup>59</sup> reported the anti-urolithiatic effect of *T. bellirica* fruits on ethylene glycol-induced Renal calculi in albino rats. Renal stone was induced in animals by 0.75 % ethylene glycol in drinking water for 28 days. The test groups administered with methanolic extract of *T. bellirica* (MeTB) at the doses 100, 200 and 400 mg/kg b.w. (p.o.) once in a day from 15th to 28th day. Cystone (750 mg/kg body weight) was used as standard drug. The effect of *T. bellirica* on various physical and biochemical parameters in urine as well as in serum was evaluated. *In vivo* antioxidant activity and lithiatic markers were estimated in kidney homogenate. The results demonstrated that metB (400 mg/kg b.w) significantly reduced the ethylene glycol induced disturbance in various physical and biochemical parameters in urine as well as in serum. metB (400 mg/kg b.w.) prevented the depletion of GSH level and decrease in the level of SOD in ethylene glycol induced renal injury from glycol induced calculi in rats.

#### Nephrotoxic effects

Possible protective effects of *T. bellerica* were investigated by Fatima and Sultana<sup>60</sup>. The rats were divided into five groups. The first group served as a control and was injected with the normal saline, second group was injected with Gentamicin (80 mg/kg IP), third group was injected with gentamicin plus standard Silymarin 100 mg/kg p.o., the fourth and fifth group animals were injected with *T. bellerica* ethanolic and aqueous extracts (500 mg/kg) plus gentamicin respectively for 15 days. GM-treated rats showed early kidney dysfunction as serum urea, uric acid and creatinine levels increased significantly. The significant decrease in GSH levels, SOD, CAT activities and increase in TBARS levels, indicated that GM-induced nephrotoxicity was mediated through oxidative stress reactions. histopathological examination of GM treated rats

revealed degenerative changes in glomeruli tubules. Administration of *T. bellerica* plus gentamicin protected kidney tissues against nephrotoxic effects of gentamicin as evidenced from amelioration of histopathological changes and re-normalization of kidney biochemical parameters.

#### Traditional uses

The leaves enhance appetite, relieves piles, lowering cholesterol and blood pressure, boosts immunity and prevents ageing. It also enhances the body resistance. It is used as popular and common traditional medicine for the above ailments by people of Coimbatore district<sup>61</sup>.

Fruits are laxative, astringent, anthelmintic and antipyretic; also useful in hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhoea, coughs, hoarseness of voice, eye diseases and scorpion-sting; used as a hair tonic. A decoction of the green fruit is used for a cough. The pulp of the fruit checks dysenteric-diarrhoea, dropsy, piles and leprosy. Seed oil is used for relieving pains of rheumatism. The gum of the bark is demulcent and purgative. The triterpenoid present in the fruits possesses significant antimicrobial activity. Kernel oil has purgative action and its prolonged use was well tolerated in mice<sup>62</sup>.

Seeds are also used as an aphrodisiac, oil extract from the seed pulp is used in leucoderma and alopecia. Modern investigations have proved the laxative activity of the oil<sup>20,63</sup>. Bibhitaki has astringent qualities and is best known for its use in treating all the cough and cold-related problems, dental problems, improving immunity, digestive, liver and eye problems and for fast healing of wounds. The oil from seeds with the fruit is well known for its use in treating premature greying and also promoting hair growth<sup>64</sup>.

#### Dosage and hints for intake

For a cough, asthma and indigestion: Take equal quantities of liquorice root, long pepper and *bibhitaki* (after removing the seed) and powder it as finely as possible. Take about 2 tsp of the powder and boil it along with 500 mL of water till it reduces to about 300 mL. Drink about 100 mL of this decoction twice a day (morning and evening). This remedy will give good relief from indigestion, asthma and cough.; For a sore throat: Take one broken shell of the fruit and suck on it, it works as an astringent having bitter taste but relieves for a sore throat and cough.; For wounds: Bibhitaki fruit shell paste is an amazing wound healer. Very useful for minor rashes and skin irritations. For paste preparation, take a small piece of *bibhitaki* and

rub it on a stone (sandalwood stone) along with small amount of water to get a smooth paste; for dental problems: decoction made by boiling the shell of the broken fruit along with 50-75 mL water and rinse the mouth. It takes care of most of the dental problems also the mouth odour, ulcers, etc.; Natural tonic: Pure *bibhitaki* shell powder can be used instead of the *bibhitaki* fruit. *Bibhitaki* powder when mixed with honey acts a natural tonic and applied as a warm water poultice it gives quick relief of minor skin irritations.

#### Micropropagation: Tissue culture investigations

Commercial exploitation of *T. bellerica* by various industries along with losses due to indiscriminate felling of trees, conversion of forest land into agriculture land and human settlements have all led to rapid depletion of its genetic resources. There is an increasing need for the conservation of these plants through systematic cultivation for maintaining germplasm for selection of desired genotypes and mass propagation of the superior clones. Conventional methods of *T. bellerica* propagation are insufficient because of poor seed germination and low survival rates of stem cuttings<sup>66</sup>. Therefore, alternative, rapid propagation of selected tree stocks is needed.

A protocol for micropropagation of plants via axillary bud proliferation from nodal explants of *T. bellirica* seedlings has been established. Explants can be cultured on Murashige and Skoog (MS) medium with different concentrations of 6-benzyladenine or kinetin. Within the range evaluated, the medium containing 13.3  $\mu\text{M}$  BA showed the highest shoot length (1.9= 0.2 cm) in the primary culture. When separated and transferred to fresh subculturing medium with lower levels of BA or Kn, the nodal segments from individual regenerants showed efficient shoot induction at 4.4  $\mu\text{M}$  BA<sup>67</sup>. Rathore *et al.*<sup>68</sup> used nodal segments obtained from 15 days old aseptically grown seedling as explants. MS medium containing 1.5 mg/L BAP was found suitable for culture initiation. Best rooting response (60 %) was observed on medium containing quarter strength MS salts, 0.6 % agar and 0.1 mg/L IBA.

Phulwaria *et al.*<sup>69</sup> found MS medium containing 2.22  $\mu\text{M}$  BAP to be the best for shoot multiplication in a single step. After excision of newly formed shoots, mother explants successively transferred to the same medium produced maximum number of shoots per explant. Further enhancement in morphogenetic response occurred when excised shoot clumps (2-3

shoots) were subcultured on MS medium supplemented with 2.22  $\mu\text{M}$  BAP, 1.16  $\mu\text{M}$  Kn and 0.57  $\mu\text{M}$  IAA. Half-strength MS medium supplemented with 24.60  $\mu\text{M}$  IBA and 100 mg/L AC was most effective for rooting of the shoots. To reduce labour, cost and time, an experiment on *ex vitro* rooting was also carried out and it was observed that highest per cent shoots rooted *ex vitro* when treated with 2,460  $\mu\text{M}$  IBA for 5 minutes.

Rapid *in vitro* micropropagation was also achieved using nodal explants from a mature tree on MS medium supplemented with 0.5 mg/L 6-benzylaminopurine showed 100 % shoot-bud with 4.5 $\pm$ 0.56 cm shoot length per explant. The nodal segments from micro shoots obtained from induction medium were cultured on MS basal medium supplemented with different concentrations of BAP and NAA. Best shoot multiplication occurred with 0.25 mg/L BAP + 0.25 mg/L NAA. The shoot and node number increased significantly in the third and fourth subculture of nodal segments. Excised shoots (2-3 cm long with 2 to 3 nodes), when grown on half MS basal medium with 0.25 mg/L with indole-3-butyric acid (IBA), has shown rhizogenesis. After four-weeks, plants were transferred from greenhouse to net house where the plants exhibited gradual acclimatization to outdoor conditions<sup>70</sup>.

Dhaker *et al.*<sup>71</sup> found MS medium containing 2.0 mg/L BAP was most suitable for culture initiation. Although shoot multiplication was achieved on MS medium containing BAP and Kn, the maximum number of shoots was obtained with 3.5 mg/L BAP + 0.5 mg/L Kn. Best rooting was observed on medium containing quarter strength MS salts, 0.8 % agar and 1.0 mg/L IBA. Maximum callus induction was observed on MS medium supplemented with 0.25 mg/L 2,4-D+ 0.3 mg/L Kn within 4 weeks from leaf petiole.

Another study reported an efficient *in vitro* micropropagation protocol from nodal segments of a 30-year-old tree. Nodal segments were taken from the mature tree in March-April and cultured on half strength MS medium. The highest multiplication of shoots was achieved on medium supplemented with 6-benzyladenine (BA, 8.8  $\mu\text{M}$ ) and  $\alpha$ -naphthalene acetic acid (NAA, 2.6  $\mu\text{M}$ )<sup>72</sup>.

#### Genetic diversity of *T. bellirica*

Dangi *et al.*<sup>73</sup> studied genetic diversity in *T. bellirica* based on morphological, phytochemical and molecular markers from 28 accessions belonging to 4 ecogeographic regions. Significant differences were observed for morphological descriptors viz., leaf type, fruit shape and plant nature. UPGMA dendrogram for

morphochemical traits clustered the accessions into three groups. Molecular diversity was studied using RAPD and ISSR markers. A total of 19 polymorphic primers (15 RAPD and 4 ISSR) produced 55 polymorphic bands with an average of 5.8 bands per primer. At 59 % similarity UPGMA derived dendrogram divided the populations into clusters based on geographical distribution. High genetic differentiation ( $G_{ST} = 0.63$ ) was found among the populations with restricted gene flow ( $N_m = 0.26$ ).

Genetic diversity was assessed within each population, among the populations and also in micro propagated plantlets of *T. bellirica* using random amplification amplified polymorphic DNA (RAPD) marker in plants collected from four different districts of Chhattisgarh State, India and ten from *in vitro* produced plantlets using Twenty primers for RAPD analysis. RAPD analysis which yielded 168 bands of which 152 polymorphic bands (90 % polymorphism), revealing a very high level of genetic diversity in the species<sup>74</sup>.

#### Cytological studies

The cytological observations revealed that mother tincture of *T. bellerica* had a strong mitostatic effect on *Vicia faba* root as evident by the mitotic index which decreases with the increases in duration from 4 to 12 hours and concentration from 5 to 50 % in the test plant. The reduction in mitotic index might be due to physiological changes induced by the drug in nuclear chromatin and chromosomal anomalies<sup>27</sup>.

#### Toxicity studies

Thanabhorn *et al.*<sup>75</sup> evaluated acute and subacute toxicities of the ethanol extract from *T. bellirica*. A single oral administration of the ethanol extract @ 5,000 mg/kg did not cause toxicity, behavioural changes, mortality and differences on gross appearance of internal organs. For the subacute toxicity, all rats received a repeated oral dose of 1,000 mg/kg of the ethanol extract over 14 days. The satellite group was given the ethanol extract in the same period but kept for further 14 days without medication to detect the delayed effects or reversibility of toxic effects. The results showed that the extract did not cause any changes in terms of general behaviour, mortality, weight gain, haematological or clinical blood chemistry parameters. The histological examinations showed the normal appearance of the internal organs very comparable to the control group.

Sireeratawong *et al.*<sup>76</sup> assessed acute and chronic toxicities of the water extract from the dried fruits of *T. bellerica* in both female and male rats. For acute

toxicity, a single oral administration of the water extract @5,000 mg/kg body weight (10 female, 10 male) was given. No toxicity (such as general behaviour changes, morbidity, mortality, changes in gross appearance or histopathological changes of the internal organs of rats) was seen. The study of chronic toxicity was determined by oral feeding both female and male rats (10 female, 10 male) daily with the test substance @3 00, 600 and 1,200 mg/kg body weight continuously for 270 days. Repeated examinations for signs of toxicity showed no abnormalities in the test groups as compared to the controls.

Acute administration of aqueous acetone extract of *T. bellirica* fruits (AATB) was done in female Wistar albino rats as a single dose up to 2000 mg/kg body weight. In the end, blood was collected for biochemical and haematological analyses, while histological examinations were performed on liver and kidney. There was no alteration in the behavioural pattern, food and water intake in the treated animals. The relative organ weight, biochemical parameters, haematological parameters and histopathological analysis were also just like the normal. All the parameters for toxicity evaluation suggest that aqueous acetone extract of *T. bellirica* fruit is safe, to be used as a traditional herbal formulation for its antioxidant potential and other health benefits<sup>77</sup>. Kuriakose<sup>78</sup> suggested that its therapeutic potential is helpful in alleviating hepatic oxidative stress and tissue damage, hence the traditional use of the plant in this regard stands justified.

#### Conclusion

An updated literature survey revealed that *T. bellirica* has a diverse pharmacological spectrum. This has been used in Ayurveda, Siddha, Chinese medicine etc, because of having important phytoconstituents viz., Gallo-tannic acid, bellericanin, ellagic acid, gallic acid, termilignan, thanni lignan, flavone and anolignan B, Tannins, ellagic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyllembin, - sitosterol, mannitol, glucose, fructose and rhamnase. Because of these compounds, it shows many of the pharmacological activities such as antisecretory, analgesic, antihypertensive, antidiarrhoeal activity, antimicrobial activity, antidiabetic, antioxidant, antiulcer, antipyretic, hepatoprotective, anticancer, angiogenesis, antidepressant-like activity. It is anti-uro lithiatic. It is useful in the treatment of gastric ulcer, constipation, general debility, piles etc. still, it has actually not been explored comprehensively. Thus it



needs to be explored the potent phytoconstituents from the plant having valuable pharmacological properties for new high-quality drug formulations.

*T. bellirica* has the antibacterial potential to derive novel antimicrobial agents for the treatment of various infections for developing new medicines. It can be exploited differentially by the pharmaceutical industry. It has antioxidant potential and many health benefits. The extracts of *T. bellirica* also has promising anticancer ingredients but the promising active principles and the underlying mechanism by which there is such activity in *T. bellirica* needs to be further investigated. *T. bellirica* is a promising angiogenic agent. *T. bellirica* and also have a nephroprotective effect. It can prevent the deposition of CaOx crystal in the kidney.

The seedling derived explants have been used for micropropagation as they are easy to establish in culture. In *T. bellerica*, MS medium containing 1.5 mg/L-2.0 mg/L BAP was the best for culture initiation while 1-2.0 mg/L BAP was most suitable for shoot multiplication. Best shooting response was observed on media containing BAP and IBA (Auxin) and widely used for root induction hormone. *T. bellirica* genotypes revealed a high level of RAPD variations in the population.

### Conflict of interest

We declare that we have no conflict of interest.

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