

Therapeutic Properties of *Trichopus zeylanicus* Subsp. *travancoricus*, a Rare, Endangered Medicinal Plant in South India: A Review

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Abstract

Trichopus zeylanicus subsp. *travancoricus*, belonging to the family *Trichopodaceae*, is a small herbaceous plant exclusively present in Western Ghats of South India. The indigenous tribal community in Western Ghats traditionally use this plant for getting instant energy to combat fatigue. Recent pharmacological studies have revealed that besides its antifatigue property, this plant possess many medicinal properties such as anti-oxidant, anti-inflammatory, anti-stress, immunomodulatory, anti-diabetic, aphrodisiac, antihyperlipidemic, antitumor, antiulcer, antimicrobial and hepatoprotective activity. This article comprehensively review the results of pharmacological studies so far done in this plant and emphasizes perspectives that warrant future research to explore its full pharmacological potential.

Keywords: India; Traditional knowledge; *Trichopodaceae*; *Trichopus zeylanicus*; Medicinal plant; Phytochemicals; Anti-fatigue; Anti-stress

Introduction

Trichopus zeylanicus (Gaertn) is a dwarf shrub belonging to the family *Trichopodaceae* [1]. Three subspecies of *Trichopus zeylanicus* (Gaertn) are known namely *Trichopus zeylanicus* subsp. *zeylanicus*, *T. zeylanicus* subsp. *angustifolius* and *T. zeylanicus* subsp. *travancoricus*. Among these sub species, the first two are endemic to Sri Lanka while *T. zeylanicus* subsp. *travancoricus* is distributed to Western Ghats, Malaysia and Thailand. So far, medicinal properties have been reported only for *Trichopus zeylanicus* subsp. *travancoricus* (correct nomenclature is *Trichopus zeylanicus* Gaertn. subsp. *travancoricus* Burkill ex Narayanan subsp. nov) [2].

In India, *Trichopus zeylanicus* subsp. *travancoricus* (hereafter called TZT) is endemic to Agastya hills, the extreme end of Western Ghats mountain range of South India. For centuries, TZT has been in use as an instant energy stimulant within Kani tribe, an indigenous tribal community settled in Agastya hills.

This medicinal property of TZT is known to the scientific world only after a publication that came in 1988 where the authors claim the instant energy property of TZT based on their direct experience by eating the fresh seeds during their expedition to Agastya hills [3]. Within the Kani community TZT is known as "Arogyapacha" literally means "the greener of health" i.e., the one that gives very good health and vitality. The traditional knowledge from the Kani tribe about TZT as a medicine paved the way for the scientific community to further explore the pharmacological potential of this plant.

A scientifically validated and standardized herbal drug named "Jeevni" had been developed from the whole plant by Indian scientists and was released for commercial production in 1995 by a Pharmaceutical firm in India [4]. While transferring the technology for the production of the drug to the pharmaceutical firm, a benefit sharing agreement was signed to a Kani trust to share 50% of the license fee and royalty with the tribal community.

This agreement between the Kani's and the scientific community which was first of its kind and is considered as a good model for using traditional knowledge from indigenous communities. A varied spectrum of pharmacological properties of TZT has been reported so far from different parts of the world. This review focuses on various pharmacological properties of TZT based on the available scientific reports and discusses the possible future research on this plant.

Methodology

The present review covers the literature available from 1989 to 2018. A systematic review was carried out in public databases such as PubMed (www.ncbi.nlm.nih.gov/pubmed) and Jstor, ScienceDirect (www.sciencedirect.com) and SciFinder (www.libnet.ulg.ac.be/en/eresources/scifinder-scholar) (www.jstor.org/) using the keywords *Trichopus*, *Trichopus zeylanicus* and *Arogyapacha*. This search resulted into identification of 182 literatures. Among these, 38 articles relevant to the scope of this review were selected and critically evaluated. The chemical structures have been revised by consulting the open chemistry database PubChem (pubchem.ncbi.nlm.nih.gov/search/#collection=compounds), and then redrawn using the freeware version of the software ACD/ChemSketch (Freeware) 14.01.

Literature Review

Botanical description of *Trichopus zeylanicus*

Trichopus zeylanicus is a small herbaceous plant usually growing along the wet banks of streams and rivulets on hills. It has many slender stems around 5 cm to 25 cm long arising from its nodose rhizome (**Figure 1**) [3,5]. There is one terminal leaf on each stem. The long petiole appears like a continuation of the stem. In general, the leaves are heart shaped, but may vary to different shapes like triangular, ovate with an obtuse apex and basally cordate with a wide sinus (**Figure 1**).

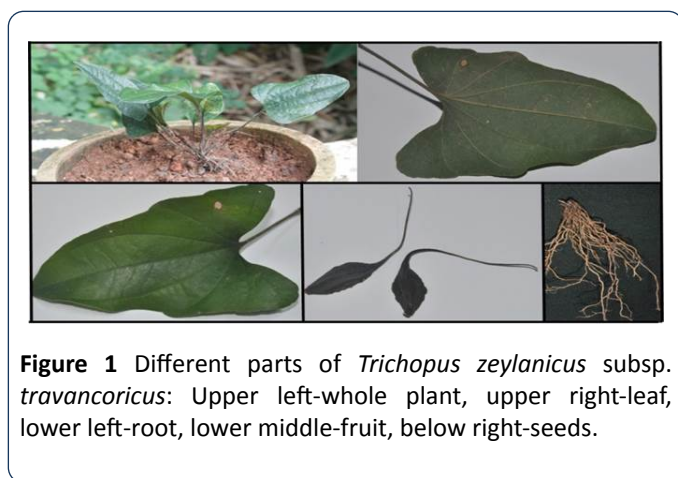


Figure 1 Different parts of *Trichopus zeylanicus* subsp. *travancoricus*: Upper left-whole plant, upper right-leaf, lower left-root, lower middle-fruit, below right-seeds.

Flowers are deep purple colored small, medium bisexual, mostly one, fascicled at the base of the leaves, extruded from between the protecting scale leaves. Perianth dark-brown, sub-equally 6-lobed, Stamen 6 with sub sessile anthers, filaments widening into broad connectives.

Ovary inferior, 3 celled with two superimposed ovules in each cell. Stigma 3-lobed. Fruits are somewhat winged, triangular and indehiscent (**Figure 1**).

The tender kernel of immature fruit is sweet to taste and has pleasant flavor. On ripening it becomes stony and unpalatable. Seeds are endoplasmic and its endosperms are ruminating (and cartilaginous); these are ovate, dorsally grooved, and rugose.

Embryo is well differentiated and straight. *Single cotyledon* is present at the tip of the axis and *the plumule* occupies the terminal position. Testa without phytomelan, very thin micropyle zigzag. It possess fibrous root system (**Figure 1**).

Taxonomical position of *TZT*

Taxonomical position of *TZT* is still in debate. It was previously assigned to family *Dioscoreaceae*. But based on the morphological and cytological dissimilarities to other species in *Dioscoreaceae*, *T. zeylanicus* was excluded from this family and now assigned to the family *Trichopodaceae* (**Table 1**) [1].

Table 1 Taxonomic position of *Trichopus zeylanicus*.

Division	<i>Mangoliophyta</i>
Class	<i>Liliopsida</i>
Order	<i>Dioscoreales</i>
Family	<i>Trichopodaceae</i>
Genus	<i>Trichopus</i>
Species	<i>zeylanicus</i>

Phytochemicals of *TZT*

Preliminary phytochemical screening of different extracts of *TZT* revealed the presence of various secondary metabolites such as phenolics, alkaloids, flavonoids, tannins, terpenoids, steroids glycosides, saponins etc., [6-8].

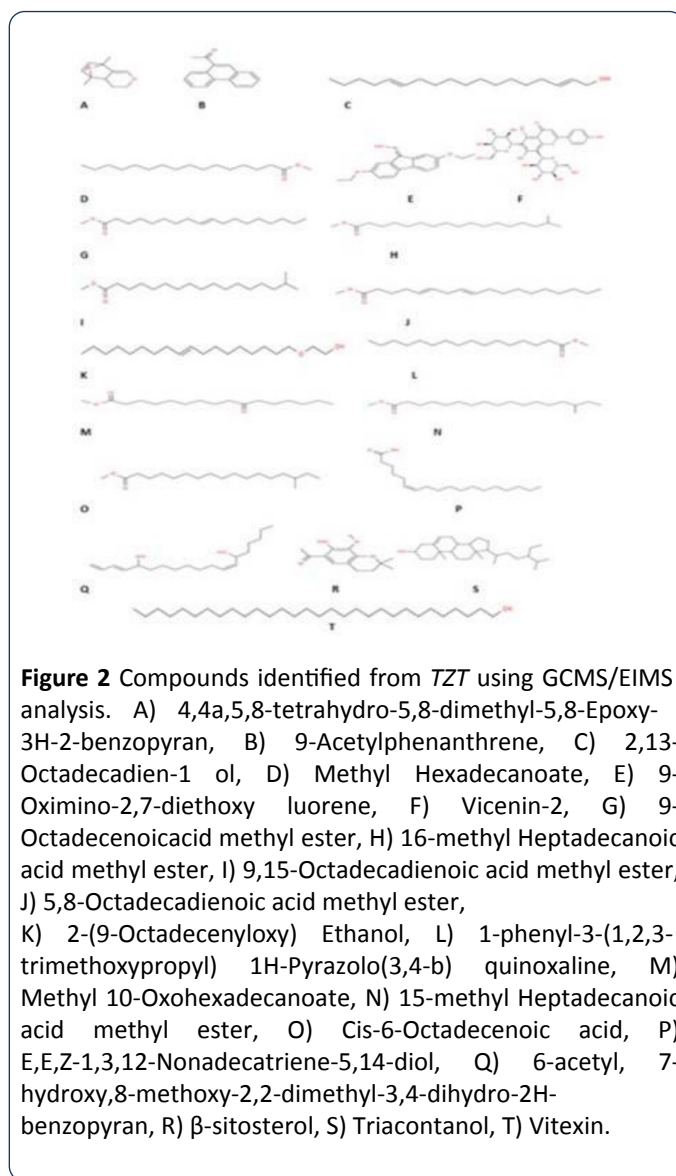


Figure 2 Compounds identified from *TZT* using GCMS/EIMS analysis. A) 4,4a,5,8-tetrahydro-5,8-dimethyl-5,8-Epoxy-3H-2-benzopyran, B) 9-Acetylphenanthrene, C) 2,13-Octadecadien-1 ol, D) Methyl Hexadecanoate, E) 9-Oximino-2,7-diethoxy luorene, F) Vicenin-2, G) 9-Octadecenoic acid methyl ester, H) 16-methyl Heptadecanoic acid methyl ester, I) 9,15-Octadecadienoic acid methyl ester, J) 5,8-Octadecadienoic acid methyl ester, K) 2-(9-Octadecenyloxy) Ethanol, L) 1-phenyl-3-(1,2,3-trimethoxypropyl) 1H-Pyrazolo(3,4-b) quinoxaline, M) Methyl 10-Oxoheptadecanoate, N) 15-methyl Heptadecanoic acid methyl ester, O) Cis-6-Octadecenoic acid, P) E,E,Z-1,3,12-Nonadecatriene-5,14-diol, Q) 6-acetyl, 7-hydroxy,8-methoxy-2,2-dimethyl-3,4-dihydro-2H-benzopyran, R) β -sitosterol, S) Triacantanol, T) Vitexin.

So far 21 compounds were identified from *TZT* through GC-MS/EI-MS analysis and are listed in **Table 1** and are shown in **Figure 2**.

Potential Therapeutic Properties of *TZT*

After the first report on the medicinal properties of *TZT* by Pushapangadan et al. [3], various experiments were carried out to explore its diverse medicinal properties and were discussed in detail below.

Antifatigue property

The antifatigue property of *TZT* has been tested in experimental rats through forced swim test, a method to evaluate fatigue/depression in animals [9]. Sharma et al., demonstrated that experimental rats treated with aqueous suspension of ethanol (50%) extracts of the seeds (100 mg/Kg) showed an increase in swimming time compared to control animals (non-administrated) in a plastic bucket filled with water [10].

Pushpangadan et al., checked the antifatigue effect of *TZT* extracted with different solvents and showed that methanol and acetone extracts at a dose of 200 mg/Kg possess significant antifatigue effect on rats during swimming performance [11]. They showed that water extract (200 mg/Kg) had no effect on swimming performance and suggested that the agent that induces the antifatigue effect is not extractable in water [11]. Singh et al., showed that a glycopeptido-lipid fraction of ethanol extract exhibited significant antifatigue effect and muscle coordination in mice subjected to swimming performance [12].

Evans et al., showed that the ethanolic extract of *TZT* in mice enhanced the utilization of free fatty acid in preference to glucose during intense exercise implying that *TZT* can be used as a potential sports medicine [13]. To exclude the possibility that the observed antifatigue property of *TZT* is due to amphetamine-mimetic activity, Tharakan et al., showed that the administration of *TZT* water suspension (500 mg/kg) to 6-hydroxydopamine lesioned rats did not show any ipsilateral rotation upon treatment with amphetamine, a central nervous system stimulant [14]. This results indicated that *TZT* combats fatigue without amphetamine-mimetic activity [15]. 6-hydroxydopamine is a neurotoxin which selectively destroys dopaminergic neurons. So far available evidences suggest that *TZT* could be a potential antifatigue drug [16].

Antioxidant property

Antioxidants are molecules that scavenge free radical and protect body from several serious diseases [17]. Free radicals are generally produced in human body from normal energy metabolic process and are generally counteracted by endogenous antioxidants [18]. But, exposure to x-rays, cigarette smoke, pollution, pesticides, and insecticides may generate excess amount of free radical in our body that create an imbalance between free radical activity and endogenous antioxidant defense system.

This free radical-antioxidant imbalance leads to condition called oxidative stress in our body that initiates many serious

diseases of aging such as cancer, cardiovascular disease, cataracts, immune system deficiency, and brain dysfunction [19]. Therefore, there is a high demand for exogenous antioxidants. Several studies have shown that plants are rich source of antioxidant compounds.

Tharakan et al., showed that *TZT* is able to inhibit hydrogen peroxide induced lipid peroxidation in rat brain homogenate, protect DNA from hydrogen peroxide inducing damage and lipoxygenase activity [14]. In another study, Velavan et al., claimed that *TZT* has significant cardio protective effects as evidenced by the reduction of isoproterenol-induced lipid peroxidation in plasma and heart tissues of experimental rats pre-treated with ethanol extract (500 mg/Kg) compared to that of controls (non-treated rats) [20].

Sindhu et al., demonstrated the antioxidant property of *TZT* in a 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay using various parts of *TZT* extracted in different solvents such as chloroform, methanol, petroleum ether, ethyl acetate and water [6]. Among these extracts, the leaf methanol extract showed highest free radical scavenging effect (IC₅₀ is 50 µg/ml) which is comparable to that of the antioxidant property of L ascorbic acid (IC₅₀ is 53 µg/ml in their study), a universally accepted antioxidant [6,21].

Anti-stress property

In this modern era, we are exposed to different kinds of stressors every day. This continuous exposure stimulates various disease states including hypertension, diabetes, peptic ulcer, immuno-suppression, reproductive dysfunctions, and anxiety, disturb sleep, depression, irritability, fatigue and lethargy. The plant derived drugs are gaining increasing popularity and are being explored for remedies of a number of disorders including stress.

To demonstrate the anti-stress property of *TZT*, Singh et al., estimated the level of corticosterone in the adrenal glands of stressed mice (constant swimming for 5 hour) treated with *TZT* ethanolic extract at doses of 250 mg/Kg and 500 mg/Kg [22]. In many species corticosterone is the major stress hormone secreted by the adrenal cortex which is involved in regulation of energy, immune reactions, and stress responses [23]. They found that the drug treatment inhibited the adrenal enlargement, a phenomenon resulting from stress, as well as a significant elevation in the concentration of corticosterone [22].

Rishikesh et al., assayed the anxiolytic activities of a saponin fraction of *TZT* by elevated plus maze method, light-dark test, and antidepressant activities were assayed by tail suspension test and force swimming test on mice [24]. They found that *TZT* has effective anxiolytic activity as evidenced by an increase in the percentage of time spent in open arm and reduced time in the dark chamber in light and dark model as well as reduced time spent in closed arm in elevated plus maze. Comparably, the saponin fraction of *TZT* shown significant dose dependent antidepressant activity in force swimming test (FST) and tail suspension test (TST) as witnessed by the decreased time of immobility when compared with control group [24].

These observations are confirmed by Raghu et al., using ethanolic extract of *TZT* at doses of 250 mg/Kg and 500 mg/Kg and shown that the drug treatment significantly reduced stress induced elevation in plasma corticosterone levels and hyperglycemia in rats [25]. This observation was contradictory to the observation by Singh et al., where they showed that the treatment of ethanolic extract of *TZT* increased the level of corticosterone in the adrenal cortex of stressed animals [22].

Therefore, more study is needed to confirm the effect of *TZT* on the corticosterone level. The Anti-stress property of *TZT* has been demonstrated by Raghu et al. [25]. In their study, stress was induced in experimental rats by restraining the animals in PVC restrainers for four hours which elevated the blood glucose and corticosterone level in stressed animal compared to that of non-stressed animals. Increased level of blood glucose and corticosterone are characteristic feature of a stress response.

Hydrochloric acid extract of *TZT* at a dose of 500 mg/Kg significantly reduced the blood glucose and corticosterone level in animals exposed to restrained stress. This anti-stress effect was comparable to that of ginseng at a concentration of 100 mg/Kg indicated that *TZT* is a potent anti-stress agent [25]. Moreover, the administration of *TZT* extract at the dose of 500 mg/kg significantly reduced stress induced anxiety in mice which was evidenced by a significant increase in the number of crossings in the EPM and light and dark model. Overall these evidences suggest that *TZT* is a potent adaptogenic agent.

Anti-microbial activity

The antimicrobial properties of *TZT* using methanol, hexane and chloroform extracts of leaf powder has been demonstrated recently by Manza and Saj and oil extract by Balasubramanian et al. [25,26]. Among these extracts, the methanol extract showed a significant dose dependent effect on the following bacterial strains; *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Clostridium tetani* [26]. It also showed significant dose dependent effect on fungal isolates; *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* sp., *Alternaria* sp., *Canadia albicans*, *Fusarium solani*, *Trichophyton mentagrophytes* and *Helminthosporium* spp [26].

The hexane extract showed moderate effects against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Streptococcus pneumoniae* [26]. The hexane extracts also showed moderate inhibition against *Alternaria* sp., *Fusarium solani* and *Trichophyton mentagrophytes* whereas Chloroform extracts proved inhibitory effects against *Alternaria* sp. and *Helminthosporium* spp. [26]. Moreover, fresh leaf oil of *TZT* showed profound effect on gram negative bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella terrigena* and some fungal organism such as *Candida glabrata* and *Candida albicans* [27].

Aphrodisiac property

Aphrodisiacs are substances that increases sexual desire when consumed. Subramoniam et al., has showed that the administration of ethanol extract of *TZT* leaf (200 mg/kg) in male mice enhanced mounting behaviour and mating performance compared to that of control animals [28]. The pups of the mice treated with the extract were found to be normal in growth, litter size and sex ratio. The water as well as *n*-hexane extracts of the plant leaf were found to be inactive. More extensive investigation should be needed to get more insight into this property or identify the actual molecule confer this effect. In addition, the effect of the drug on female sexual behaviour and fertility remains to be investigated.

Analgesic and anti-inflammatory property

An analgesic or painkiller is any member of the group of drugs used to relief from pain without loss of consciousness. In many diseases such as cancer there is a huge need for rapidly acting, powerful "rescue" analgesic which has no other side effect. To demonstrate the analgesic property of *TZT*, Sambath et al., has showed that treatment of an alkaloid fraction of *TZT* (AFTZ) in mice significantly reduced the number of writhing induced by 0.6% acetic acid in a dose dependent manner [8,24]. In another experiment, they used hotplate method in which they placed the drug treated mice on a hot plate of constant temperature of 55°C and found that the drug treatment significantly reduced the mean time of basal reactions such as flick the paw or jump from the hot plate compared to that of non-treated animals.

Inflammation is a body's immune response to heal the wound after an injury by defending itself against foreign invaders, such as viruses and bacteria and repair damaged tissue. However, it can be problematic as it plays a major role in many chronic diseases such as rheumatoid arthritis. Hence, proper treatments are to be taken against it. Subramoniam et al. showed that *Trichopus zeylanicus* leaf extract has the ability to stabilize mast cells, a type of white blood cell that contains many granules rich in histamine and heparin [29].

To demonstrate anti-inflammatory property of *TZT*, Singh et al., induced acute edema using 1% carrageenan in one of the hind paws of experimental rats prior treated with a glycol-peptido-lipid fraction of ethanol extract of *TZT* at doses of 12 mg/kg, 25 mg/kg, 50 mg/kg and 100 mg/kg and found that the drug inhibited the induced edema in a dose dependent manner [30]. The glycol-peptido-lipid fraction of ethanol extract of *TZT* was also found to be effective against adjuvant induced polyarthritis in rats [12]. Similarly, Sambath et al., showed that the treatment of an alkaloid fraction of *TZT* (AFTZ) significantly inhibited paw edema induced by carrageenan in mice in a dose dependent manner which is comparable to that of the effect shown by diclofenac sodium, a standard anti-inflammatory drug [8].

Immunomodulatory property

Immunomodulators are drugs which either suppress or stimulate immune system. As an evidence for the immunomodulatory property of *TZT*, Pushpangadan et al. had demonstrated that the *TZT* whole plant powder water suspension treatment for seven consecutive days markedly increased the proliferation of thymocytes, splenic lymphocytes, total blood leucocytes and peritoneal macrophages, the cells played a major role in cell mediated immunity [11]. They hypothesized that the drug may act on immunity specific cell because the drug treatment had no effect on Haemoglobin content, liver and body weight. Immunomodulatory activity of alkaloid fraction of *TZT* was evaluated by Rishikesh et al., in a delayed type hypersensitivity test (DTH) [31].

In their study, mice were immunized by injecting 20 μ l of 0.5×10^9 Sheep Red Blood Cells (SRBC) into right foot pad to induce foot paw edema, then treated with the alkaloid fraction of *TZT* for 14 days and on the 14th day the animals were challenged by 20 μ l of 0.025×10^9 SRBC into left foot pad. They found that the drug enhanced delayed type hypersensitivity (DTH) reaction evidenced by the significant reduction of foot paw edema as compared to control group. In this study they also showed that the drug treatment significantly increased the essential immune cells such as neutrophils, WBCs, RBCs, and Hb indicating the immune modulatory effect of *TZT* [31].

Anti-tumor property

Puspangadan et al., had demonstrated the potent antitumor effect of *TZT* [11]. In their study, mice treated with *TZT* whole plant powder water suspension (0.5 ml of 2% suspension/mouse) for 7 consecutive days were challenged with Ehrlich Ascitic carcinoma (EAC) cells (0.5 million cells/mouse) in the peritoneal cavity. After the challenge, the treatment continued for another 20 days.

The examination of peritoneal cavities of mice after the 20 days revealed that the drug treatment completely protected 60% of mice from the tumor cell growth and the number of tumor cells were dramatically reduced in treated mice. In tumor control mice (drug-untreated), full tumor growth was observed in all animals. Even though the mechanism behind the anti-tumor property is not understood, they also observed that the drug treatment dramatically increased polymorphonuclear leucocytes and peritoneal Macrophages, the most important phagocytic cells, as compared to total leucocyte in drug treated mice.

Antiulcer property

The effect of fresh seed ethanol extract on gastric ulceration induced by restraint, cold and aspirin was evaluated by Sharma et al. [10]. They found that the drug pre-treatment in mice significantly reduced the incidence and severity of ulcer induced by aforementioned methods compared to that in controlled mice where the incidence of ulcer was 100%.

Furthermore, Singh et al. had showed that pre-treatment with alkaloid fraction of ethanol extract of *TZT* significantly reduced gastric ulceration and its severity in mice subjected to forced swim and immobilization, respectively, as compared to that in controlled animals (non-treated) [12]. In a recent study Rishikesh et al., demonstrated that saponin fraction of *TZT* showed a dose dependent effect in lowering gastric ulcer induced by ethanol and restrained stress and pyloric ligation induced ulcer in rats [32].

Anti-hyperlipidemic property

Hyperlipidemia is abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It is a risk factor of coronary heart disease due to their influence on atherosclerosis. Recent experiments by Reddy et al., showed that *TZT* possess potential antihyperlipidemic property [33]. They showed that in high fat Diet and Triton X-100 induced hyperlipidemic rats, the administration of methanolic extract of *TZT* at a dose of 400 mg/kg/day caused a significant decrease in the levels of serum cholesterol, triglycerides, LDL, VLDL and a gradual increase in the level of serum HDL compared to untreated hyperlipidemic rats. They also observed that the hyperlipidemic effect of *TZT* was comparable to that of lovastatin, an effective antihyperlipidemic drug, used in this study as a standard.

Hepatoprotective activity

Hepatoprotection or antihepatotoxicity is the ability to prevent damage to the liver. *TZT* extract has been evaluated for its antihepatotoxic and choleric activities in rats by Subramoniam et al. [34]. The plant leaf suspension (1000 mg/kg; wet weight) as well as its methanol extract showed a remarkable hepatoprotective activity against paracetamol-induced hepatotoxicity as judged from the serum marker enzymes, liver histology and levels of lipid peroxides in liver. The effect of the methanol extract was found to be concentration dependent. They also showed that the water and hexane extracts did not showed any hepatoprotective activity. Palaniswami et al. showed that *TZT* leaf extract has the ability to attenuate the liver damage caused by $HgCl_2$ in rats [35].

Antidiabetic property

The antidiabetic property of *TZT* has been investigated by Rajan et al. in streptozotocin induced diabetic rats [7]. The treatment with *TZT* ethanolic extract at a dose of 400 mg/kg for 15 days significantly reduced blood glucose level and body weight compared to that of control animals (non-treated). The observed effect was comparable to that of Glibenclamide (0.5 mg/kg), an effective oral hypoglycemic drug, used in this study as a standard [36].

Toxicity Studies

The fruits of *TZT* is edible and traditionally use by Kani tribes to combat fatigue. Now a days, local Kani peoples drink water

boiled with *Trichopus* and no toxic effect was also been reported (Personal communication). Toxicity of saponin fraction, methanol extract and ethanol extract has been tested in Male Swiss albino mice, Adult male wistar rats and female wistar albino rats by administrating the test drug orally at one dose level of 2000 mg/kg b. w. No toxicity and mortality was reported by observing the animals periodically up to 24 hours after the treatment [24,32,33].

Discussion and Future Perspectives

In vitro and *in vivo* studies using different extracts had revealed that *TZT* is a high valuable medicinal plant with diverse medicinal properties (Tables 2 and 3). These evidence based medicinal properties of *TZT* warrant further research on this plant to utilize it as a potential drug for many human dreadful diseases. The clinical trials in humans are completely absent and are necessary to support the present findings and to the development and optical efficacy of the drug.

Even though, phytochemical screening of ethanol/methanol extracts revealed the presence of various phytochemicals such as alkaloids, flavanoids, tannins, terpenoids, steroids, glycosides, saponins etc., the mechanism or specific compound that confer its medicinal properties are hitherto unidentified. So far 21 compounds were identified from *TZT* using advanced analytical methods (Table 2). Further phytochemical screening of these compounds against various pharmacological targets will reveal more insight into the mechanism behind the medicinal properties of this plant.

There are many limitations in the proper pharmaceutical exploitation of this valuable plant. It is an endangered plant with limited distribution in India. Overexploitation of this plant for its medicinal value may lead to its extinction. Therefore, proper biotechnological approaches should be taken for preservation of this plant either *ex-situ* or *in-situ*. Plant micro-propagation is a biotechnological approach for the conservation of plant species which are under extinction.

Table 2 Compounds identified from *TZT* through GC-MS/EI-MS.

Source	Extract/Method	Compound	Class of Compound	Reference
Fresh leaves	Oil/GC-MS	5,8-Epoxy-3H-2-benzopyran 4,4a,5,8-tetrahydro-5,8-dimethyl	Substituted Benzopyrans	Balasubramanian et al. [27]
		9-Acetylphenanthrene	Substituted Phenanthrenes	
		2,13-Octadecadien-1-ol	Unsaturated alcohol	
		Hexadecanoic acid methyl ester	Saturated Fatty acid ester	
		9-Oximino-2, 7-diethoxyfluorene	Substituted Fluorenes or polycyclic aromatic hydrocarbons	
		9-Octadecenoic acid methyl ester	Unsaturated Fatty acid ester	
		Heptadecanoic acid 16-methyl, methyl ester	Saturated Fatty acid ester	
		9,15-Octadecadienoic acid methyl ester	Unsaturated Fatty acid ester	
		5,8-Octadecadiyonic acid, methyl ester	Unsaturated Fatty acid ester	
		Ethanol, 2-(9- octadecenyloxy)	Unsaturated ether	
		18,19-Secoyohimban-19-oic acid 16-methyl-, methyl ester,	Alkaloid derivatives	
1H-Pyrazolo(3,4-b) quinoxaline 1-phenyl-3-(1,2,3-trimethoxypropyl)	Alkaloid derivatives			
Dried leaves	Oil/GC-MS	Hexadecanoic acid, methyl ester	Saturated Fatty acid ester	Balasubramanian et al. [27]
		Methyl 10-Oxohexadecanoate	Saturated Fatty acid ester	
		9-Octadecenoic acid, methyl ester, (E)	Unsaturated Fatty acid ester	
		Heptadecanoic acid, 15-methyl, methyl ester	Saturated Fatty acid ester	
		6-Octadecadienoic (Z) Petroselinic acid	monounsaturated omega-12 fatty acid	
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Unsaturated Diol			
Whole plant	Hexane/EI-MS	6-acetyl-7-hydroxy,8-methoxy-2,2-dimethyl-3,4-dihydro-2H-I benzopyran	Substituted Benzopyrans or chromenes	Evans et al. [13]

Hexane/IR spectrum	B-sitosterol	Phyto sterol
Hexane/NMR spectrum	triacontanol	Fatty alcohol
Methanol/NMR spectrum	Vicenin -2	Flavone glucoside or glucosyl flavanoid
Methanol/NMR spectrum	Vitexin	Flavone glucoside or glucosyl flavanoid

Table 3 Over view of *in vitro* and *in vivo* studies on TZT.

Pharmacological Activity	Reference	Plant Part Used	Extract/Fraction used	Method/ Analysis	Experimental animals/ organism	Significant dose
Antifatigue	Sharma et al. [10]	Seed	Ethanol extract, Fresh seed paste suspension	Swimming Endurance Test	Adult male Charles-Foster rats (100-150 g) and Swiss albino mice (25-30 g)	100 mg/kg
	Tharakan et al. [15]	Whole plant	dried whole plant powder aqueous suspension	Swimming Endurance Test on young and aged animals, test for rotational behaviour	Male Sprague-Dawley rats (200-250 g), Ames dwarf mice (old)	250 mg/kg (young rat), 500 mg/Kg (old mice)
	Evans et al. [13]	Dried Leaf	Ethanol extract suspended in 5% tween 80	Swimming exercise and blood test for glucose, free fatty acid (FFA), pyruvic acid (PA) and lactic acid (LA)	Male Swiss albino mice (27-30 g)	100 mg/kg
	Pushpangadan et al. [11]	Whole plant	Methanol, ethanol and water extracts	Swimming Endurance Test	Adult male Swiss albino mice (25-30 g), Charles Foster rats (100-150 g)	250 mg/Kg (Methanol or Acetone extract)
	Singh et al. [12]	Whole plant	Glyco-peptido-lipid fraction	Swimming endurance test	Charles Foster rats (150-180 g) and Swiss albino mice (25-30 g)	25 mg/kg
Antioxidant	Sindhu et al. [6]	Dried leaves, root and fruits	Plant parts extracted using petroleum ether, chloroform, ethyl acetate, methanol and water	Phytochemical analysis and DPPH free radical scavenging assay	No animal study	Dose dependent
	Tharakan et al. [15]	Whole plant	Aqueous suspension of whole plant powder	DPPH and ABTS free radical scavenging assay. Test for lipid peroxidation effect, lipoxygenase activity, DNA protection and divalent metal chelation	Male Sprague-Dawley rats (200-225 g)	Dose dependent
	Velavan et al. [20]	Dried leaves	Ethanol extract	Test for lipid peroxidation effect	Male Wister albino rat	500 mg/kg
Anti-stress	Ram et al. [25]	Dried leaves	Ethanol extract	Evaluation for behavioural changes in stressed animals using Elevated plus maze model, open field test and Light and dark model	Male, Swiss albino mice (20-30 g), male Wister albino rats	500 mg/kg

	Rishikesh et al. [24]	Whole plant	Saponin fraction	Anxiolytic activities of Saponin fraction of TZT were tested by elevated plus maze method, light-dark models, and antidepressant activities were evaluated by tail suspension model and force swimming test on mice	Male Swiss albino mice weighing 25-30 g	Dose dependent (maximum dose used was 300 mg/kg)
	Singh et al. [22]	Whole plant	Ethanol extract	Swimming endurance test, Estimation of adrenal corticosterone	Male Swiss albino mice	500 mg/Kg
Antimicrobial activity	Manza et al. [26]	Dried leaves	Extract of Hexane, Chloroform, Methanol	Filter paper disc diffusion method	Bacteria and fungi	Dose dependent (Maximum & effective dose - Methanol extract 3 µg/ml)
	Balasubramanian et al. [27]	Dried leaves	Oil extract	GC-MS analysis antibacterial and antifungal test	Bacteria and fungi	100 µg/ml
Aphrodisiac property	Subramoniam et al. [28]	Dried leaves	Water, ethanol and hexane extract	Test for mounting behaviour and assessment of mating performance	Adult Swiss mice (25-35 g)	Ethanol extract 200 mg/kg
Analgesic and Anti-inflammatory property	Kumar et al. [8]	Whole plant	Alkaloid fraction (Methanol extract)	Phytochemical screening, Test for analgesic effect-Acetic Acid Induced Writhing Method, Hot plate method, anti-inflammatory effect - Carrageenan-induced paw edema, Cotton pellet induced granuloma	Male Swiss albino mice weighing 25-75 gm, Adult Albino rats (Wistar strain) of either sex weighing between 120-200 gm	300 mg/kg
	Singh et al. [12]	Whole plant	Glyco-peptido-lipid fraction from alcoholic extract	Hypoxia test, carrageenan-induced trauma, gastric ulceration test, analgesic test, test for hypothermic effect	Charles Foster rats (150-180 g) and Swiss albino mice (25-30 g)	Dose dependent
Immunomodulatory property	Rishikesh et al. [32]	Whole plant	Alkaloid fraction (Methanol extract)	neutrophil adhesion test, delayed type hypersensitivity reaction, and effect on hematological parameters	Male Swiss Albino mice (25-30 g)	300 mg/Kg

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