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Review Article

Can *Guduchi* (*Tinospora cordifolia*), a well-known ayurvedic hepato-protectant cause liver damage?

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ABSTRACT

Ayurveda is a centuries old traditional medicine practiced in India even today. There are certain safe medicinal plants with well-established medicinal properties both in clinical practice as well as in modern scientific publications. *Guduchi* or *Tinospora cordifolia* (Willd.) Miers (Menispermaceae), is one such medicinal plant that has well known anti-inflammatory, immune-modulatory and other safe therapeutic applications including hepato-protection, because of which it was recommended by the Ministry of AYUSH, Government of India to be used in COVID-19 care. Therefore, Aabha Nagral's article "Herbal Immune Booster-Induced Liver Injury in the COVID-19 Pandemic-a Case Series," published in 2021, was unanticipated. The article recounted histologically documented clinical cases of six patients who developed drug-induced autoimmune-like hepatitis after reported consumption of Guduchi or Guduchi containing formulations during the COVID-19 pandemic. Since the Ayurveda practitioners vouch by the safety of *T. cordifolia* (TC), it was felt that the story needed to be further scrutinized.

This article reviews the botanical entities, the substitutes and adulterants of species used as *Guduchi*, their pharmacological and toxicological properties. While the authentic botanical entity of *Guduchi* is TC, *Tinospora sinensis* and *Tinospora crispa* are also commonly traded in the Indian subcontinent as *Guduchi* or *Giloy*. Among these species, *T. crispa* is known to induce heapto-toxicity. In Nagral's article, there were variations in the reported six cases in terms of patient history and TC/TC product consumption. More importantly, the botanical authenticity of the consumed products was not investigated.

A review of published literature indicates that it is unlikely that the authentic TC could have induced autoimmune-like hepatitis of the patients. It is probable that a wrong species was self-administered by the patients. It is worth following up with the cases (patients), to investigate details of the products, so that other consumers do not suffer. Nagral's article however does highlight the serious issue of adulteration in herbal markets and the need for establishing a robust pharmacovigilant system in India.

1. Introduction

Only ~5% of the world's 3,00,000 plant species have been scientifically investigated for therapeutic purposes [1]. The popularity of herbal products globally is because they are perceived as being safe, effective, and natural even though not rapid in action. Currently, there are no effective drugs against the COVID-19 pandemic caused by the SARS-Cov2-virus [2]. Traditional Medicines are being researched extensively, especially in China, India, and other South Asian countries, in COVID-19 clinical trials due to their established anti-viral, anti-inflammatory, and immuno-modulatory properties [3]. India has a rich traditional medical

Abbreviations: AYUSH, Ayurveda, Yoga, Unani, Siddha & Homeopathy; API, Ayurvedic Pharmacopoeia of India; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; AST, Aspartate aminotransferase; BCE, Before common (or current) Era; BHT, Butylhydroxytoluene; CL, *Curcuma longa*; COVID-19, Corona Virus Disease-19; COX, Cyclooxygenase; DNA, Deoxyribonucleic Acid; DPPH, 2,2- diphenyl-1-picryl-hydrazyl-hydrate; GM-CSF, Granulocyte-macrophage colony-stimulating factor; hs- CRP, High-sensitivity C-reactive Protein; IL-6, Interleukin 6; LOX, Lipooxygenase; MT, Metric Ton; NS, No Scientific; PTBD, Percutaneous transhepatic biliary drainage; SOC, Standard of Care; TC, *Tinospora cordifolia*; TNF α, Tumour Necrosis Factor Alpha.

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system, including Ayurveda, Yoga, Unani, Siddha, Homeopathy (AYUSH), Sow-rig-pa and Naturopathy. It also has undocumented local health traditions that are passed on orally from one generation to another. The AYUSH Ministry of the Government of India has councils for each of the systems for dedicated action on research, education, regulation, and outreach.

Aabha Nagral's article "Herbal Immune Booster-Induced Liver Injury in the COVID-19 Pandemic-a Case Series", published in June 2021, presented the histologically documented experience of six patients who developed drug-induced autoimmune-like hepatitis after consuming *Guduchi* [4]. *Guduchi* is botanically known as *Tinospora cordifolia* (Willd.) Miers (Family: Menispermaceae). This plant has been promoted by the Ministry of AYUSH, Government of India, as a preventive home remedy against the ongoing COVID-19 pandemic [5]. *Guduchi* is a popular medicinal plant, a Rasayana, of the Indian Systems of Medicine. It is well-documented as a hepatoprotectant and an immune-modulating agent in the ancient *Materia Medica* of Ayurveda and Siddha [6]. Rasayanas are materials and methods used in Ayurveda to increase health and lifespan [7]. *Guduchi* is a Sanskrit word that means "something which protects the body from diseases" [8]. Therefore, it becomes pertinent to understand better the context and usage of *Guduchi* in the hepatotoxic adverse effects in cases reported by Nagral et al.

This article reviews the botanical entities used as *Guduchi*, their properties, and actions in terms of safety and pharmacological efficacy. It looks at the Ayurvedic properties and actions of *Guduchi* that are documented in the pharmacopeias. It analyses the case series details reported by Nagral *et al.* (2021) and presents the gaps that need to be addressed.

2. Historical and traditional usage

T. cordifolia (TC) or *Guduchi* as it is called in Sanskrit, has been documented as a major drug of use in the early treatises of Ayurveda, -about 3000 BCE [6]. *Guduchi*, also known as *Amrita* (meaning elixir of life), is renowned for its capacity to prevent human suffering and ailments as well as impart youth, energy, and longevity. *Guduchi*'s properties and uses are mentioned in almost all classical Ayurveda books, including the *Samhitas* and *Nighantus*. The *Nighantus* detail TC's qualities, characteristics, pharmacological activities, dosage forms, and doses [9]. *Guduchi* is described in the treatment of various disorders in Sushruta Samhita, under *Tikta-Saka Varga,* including *Svasa* (asthma), *Maha Jvara* (fever), *Aruci* (anorexia), and *Kustha* (leprosy). There is strong evidence for its use in the treatment of *Jvara* (fever), *Vata Rakta* (arthritic conditions), and *Kamala* (jaundice) in Ashtanga Hridaya and Charaka Samhita [10]. The Ayurvedic pharmacodynamic properties (*Rasapanchaka*) of *Guduchi*, as mentioned in the Ayurvedic Pharmacopoeia of India [11] are:

Taste (*Rasa*): Bitter (*Tikta*) and Astringent (*Kashaya*)
Properties (*Guna*): Light (*Laghu*)
Potency (*Virya*): Hot (*Ushna*)
Post-digestive effect (*Vipaka*): Sweet (*Madhura*)
Actions (*Karma*): *Balya* (Imparts Strength/immune-modulator), *Dipana* (digestive), *Rasayana* (enhances life), *Tridoshahara* (pacifies all doshas), *Raktashodaka* (purifies blood), *Jvaragna* (antipyretic)

Ayurveda has documented many TC dosage compositions, as well as their distinct methods of preparation and doses. These include *Swarasa* (fresh stem juice: 10–20 ml/day), *Kalka* (fresh stem paste: 10 g/day), *Churna* (powdered dry stem: 1–3 g/day), *Kwatha* (hot water extract from ground dried stem: 20–30 ml 2–3 times a day), *Phanta* (hot water infusion: 10–20 ml/day), *Arishta* (self-generated alcohol, 25 ml twice a day), *Satwa* (a starchy stem extract: 750 mg - 2 g/day), *Ghana* (crystallized aqueous extract: 500 mg - 1 g; 3–4 times/day), fat-based formulations of TC processed in ghee or oil (*Guduchi Ghruta*: 10–20 g/day) and *Guduchi Taila* (for external application). Ayurvedic texts have documented multi-ingredient combinations containing TC, some of which are widely used by practitioners. e.g., *Chandraprabhavati, Kaishor Guggulu, Abhayadi Kwatha, Rasnadi Kwatha, etc* [12,13].

Rasayana (rejuvenation therapy), is one of the important branches of Ayurveda, and it relates to nutrition and nourishment. *Guduchi* is one of the important Rasayana plants, just like *Amla* (*Phyllanthus emblica* L.), *Haritaki* (*Terminalia chebula* Retz.), and *Shunti* (*Zingiber officinale* Roscoe). *Guduchi* is also acknowledged as an elixir by modern scientific research because of its wide ranging curative and health promoting properties.

3. Habit & habitat

TC is a large smooth deciduous climbing vine found in India, China, Burma, and Sri Lanka (Fig. 1). The plant has been spotted in the tropics of Africa and Australia. It occurs Pan-India from Kumaon Mountains in the north to Kanyakumari, India's southernmost point [13,14].

The Ayurvedic Pharmacopoeia of India (API) correlates the botanical identity of *Guduchi* to *Tinospora cordifolia* (Willd.) Miers (Family:Menispermaceae) [11]. It is a large, deciduous, extensively spreading, climbing shrub with several elongated twining branches. Branches also produce copious long, filiform, pale brown, fleshy aerial roots hanging down like strings. Stems are greenish, smooth with thin greyish papery peeling, and with many small roundish protuberances called lenticels. Leaves are simple, alternate, and exstipulate with long petioles up to 15 cm long, roundish and pulvinate, both at the base and apex, with the basal one longer

Fig. 1. *Tinospora cordifolia* climber.

and twisted partially and halfway around. It gets its name **Heart-leaved moonseed** by its heart-shaped leaves and its reddish fruit. Lamina is broadly ovate or ovate cordate, 10–20 cm long and 8–15 cm broad, seven nerved, and deeply cordate at base, membranous, with a prominent reticulum beneath (Figs. 1–3). Flowers are unisexual, small on separate plants, and appear leafless, greenish-yellow on axillary and terminal racemes. Male flowers are clustered, but female flowers are usually solitary racemes. It has six sepals in two series of three each. The outer ones are smaller than the inner ones. It has six petals which are smaller than sepals, obovate, and membranous. Fruits aggregate in clusters of one to three. They are globose, smooth drupelets on thick stalks with subterminal style scars, red when ripe [15].

Guduchi is known by various names in different regions of India: *Amrita* in Sanskrit, *Tippa teega* in Telugu, *Seenthilkodi* in Tamil, *Amruthaballi* in Kannada, *Giloy* in Hindi, *Garo* in Gujarati, *Gulvel* in Marathi, *Chittamrithu* in Malayalam [16]. The dried stems of TC are sold in the markets in the local vernacular as *Guduchi*/*Giloy*/*Seenthilkodi*/*Amruthaballi etc*.

According to API (2001), the raw drug "occurs as pieces of varying thickness (0.6–5 cm diameter); young stems are green with smooth surfaces and swelling at nodes, while older ones show a light brown surface marked with warty protuberances due to circular lenticels; transversely smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues and tastes bitter" (Fig. 2).

TC belongs to the family Menispermaceae, which has about 73 genera and 350 species, chiefly found in tropical areas extending to N. America and temperate Asia [17]. The genus *Tinospora* has 32 species globally of which five species occur in India. *T. cordifolia* (Willd.) Miers. ex Hook. f. & Thomson, *Tinospora sinensis* (Lour.) Merr., *Tinospora crispa* (L.) Miers. ex Hook. f. and Thomson, *T. glabra* (Burm. f.) Merr. and the recently described *T. formanii* Udayan and Pradeep are the five main species found on the Indian subcontinent. *T. cordifolia* and *T. sinensis* are the two known therapeutic species [18].

4. Trade

Giloy is among the top-traded raw drugs in India, with >1000 MT per annum being sold in the markets [19]. The dried stems of TC are commonly traded in the raw drug markets of India as *Guduchi*/*Giloy* in the vernacular names, costing ~ Rs. 50 per kg. However other species of *Tinospora*, such as *T. sinensis* and *T. crispa* are knowingly or unknowingly substituted/adulterated with *T. cordifolia* [20]. Botanically they are different (Table 1; Figs. 3 and 4); yet to the untrained, the morphology of the traded stems of TC and other *Tinospora* species looks similar and requires other scientific techniques such as chromatography, to authenticate them [21]. In the raw drug markets of Kerala, in addition to the stems of TC, *T. sinensis* (syn. *T. malabarica*) have also been reported as being traded as *Amrita* [22]. *T. crispa* is a species found mainly in the North-Eastern region of India and is also used in South-East Asian countries. There are reports that tribes in Maharashtra (Khandesh district) use *Pergularia daemia* (Apocynaceae) as TC due to the similar morphology of dried samples [23]. Using the DNA barcoding technique, Santhosh Kumar *et al.* (2018) reported that 20% of the stems of *Guduchi* that are traded in the raw drug markets of southern India are not authentic TC but substitutes/adulterants [24].

Fig. 2. Dried stems of TC sold as *Giloy* in raw drug markets.

5. Phytoconstituents

The phytochemical composition of TC is comparable to that of *Tinospora sinensis* but different from those in other traded substitute/adulterant species.

TC is a remarkable source of nutrients, minerals, and other phytochemicals, in each part of the plant, including the leaves, stems, fruits, and roots as well as fat, protein, dietary fibers, calcium, and other nutrients. TC is rich in alkaloids (Berberine, Choline, Palmatine, Tembetarine, Magnoflorine, Tinosporin, Isocolumbin), Glycosides (Tinocordiside, Cordioside), Diterpenoid (furanolactone), steroids (beta-sitosterol), aliphatic compound (octacosanol), and others (giloin, tinosporic acid) [1,25] (*T. sinensis* contains Tinosenin, berberine, 4-methyl-heptadec-6-enoic acid ethyl ester, 3 hydroxy-2, 9, II-trimethoxy-5,6-dihydro isoquinol [26]. *T. crispa* contains flavonoids (Apigenin, Diosmetin), triterpene, diterpene glucoside, cisclerodane, alkaloids (berberine, magnoflorine, higenamine), lignin, sterol [27].

6. Pharmacology and toxicology

The pharmacological actions relevant to COVID-19 disease were reviewed as below. A summary of the same has been presented in Table 2.

6.1. COVID- 19

COVID- 19 is caused by severe acute respiratory syndrome coronavirus-2 (SARS-Cov 2). Its primary entry route is through the nasopharyngeal route to the upper respiratory tract, progressing further to the lungs. Symptoms of cold, cough, fever, and breathlessness reflect viral multiplication, inflammation, the body's immune response, and low oxygen levels. It causes a cytokine storm, *i.e.,* a protracted inflammatory reaction in the lungs that rapidly deteriorate the oxygen-carrying ability [28]. As of date, there are no proven drugs available for the prevention/cure of COVID-19 disease. Due to its well-known safe use in traditional medicines (API, 2001) and published scientific literature on anti-viral effects [29], TC has recently caught the attention of anti-COVID-19 drug researchers and pharmaceutical industries across the world [30].

The Ministry of AYUSH advised different "self-care" instructions during the "COVID-19 outbreak," including health prevention strategies through improved immunity, particularly for respiratory health [5]. Based on the COVID-19 symptoms, the AYUSH Ministry came up with simple home-based remedies that were well-known immunity boosters in AYUSH systems and promoted them across India, so that the body's immunity could fight the SARS-Cov2-virus. *Guduchi* is one such plant drug with excellent immuno-modulatory properties that can boost natural immunity towards COVID-19

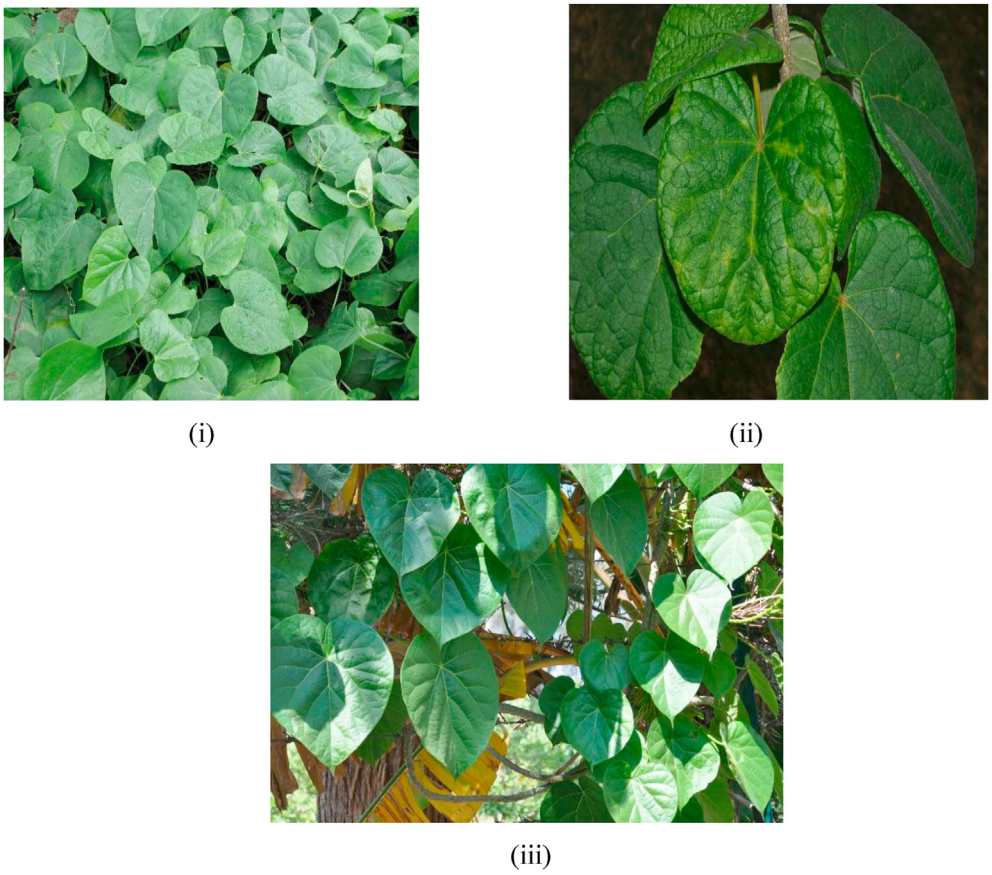

Fig. 3. Leaves of (i) *Tinospora cordifolia,* (ii) *T. sinensis* and (iii) *T. crispa.*

Table 1
Morphological characteristics of *Tinospora cordifolia* and its substitutes and adulterants [15].

Morphological Characters	*Tinospora cordifolia*	*Tinospora sinensis*	*Tinospora crispa*
Leaves	Leaves broadly ovate-cordate, glabrous, usually domatia present at the lower side, membranous	Ovate to suborbicular, pubescent above and pilose beneath, domatia usually absent	Broadly ovate to oblong orbicular, glabrous, domatia absent
Flowers	Petals 6. Male flowers on *ca* 5 mm long pedicels	Petals 6. Male flowers on *ca* 5 mm long pedicels	Petals usually 3. Male flowers on *ca* 10 mm long pedicels
Fruit (Drupe)	Globose, red when ripe	Globose, red, scarlet, or orange-red when ripe	Ellipsoid, orange when ripe
Stem	Not tuberculated, glabrous	Not tuberculated, hairy atleast young stems	Strongly tuberculated, glabrous
Distribution	Throughout India, Sri Lanka, Bangladesh, and Myanmar	Throughout India, Sri Lanka, Bangladesh, Nepal, Myanmar, China, Thailand, Cambodia	Eastern India, China, Cambodia, Thailand, Malaia, Java, and the Philippines
Sap	Watery	Watery	Milky

infections [31]. TC was selected for COVID-19 prevention, also due to the published scientific literature on its anti-oxidant, anti-inflammatory, anti-viral, immunomodulatory properties, and safety profile. Copper, calcium, phosphorus, iron, zinc, and manganese are valuable micronutrients present in *Guduchi* [32]. Traditionally TC is used as a *Rasayana* (health promoter) and a hepato-protectant in conditions like jaundice (*Kamala*). It is legally recognized for medical use and included in Indian Pharmacopoeia, which is a compendium of regulated modern drugs [33]. An overview of pharmacological activities of TC that are pertinent to COVID-19 treatment and safety studies have been presented.

A critical analysis of the AYUSH studies registered in the Clinical Trials Registry of India during the early stages of the COVID-19 pandemic period revealed that TC was the maximum featured drug for COVID-19 management [34]. Traditional Ayurvedic classical formulations of TC and *Piper longum* L. (Pippali) as an add-on to the standard of care (SOC) were compared to SOC alone in an exploratory nonrandomized prospective trial. This study was conducted on mild - to - moderate COVID-19 cases – at a tertiary care integrative medical center in the National Capital Region, Gurgaon, India. Results of this study show that the Ayurvedic add-on formulation of TC and *P. longum* shortened hospital stay and improved recovery time. In the 3-month follow-up after discharge, the Ayurveda add-on group had a better overall feeling of wellbeing and activity levels [35]. A randomized placebo-controlled pilot clinical study conducted on mild to moderate COVID-19 patients using Ayurvedic regime that included 1 g *Guduchi ghan vati* (TC tablet) showed faster and better recovery, SARS-Cov 2 viral

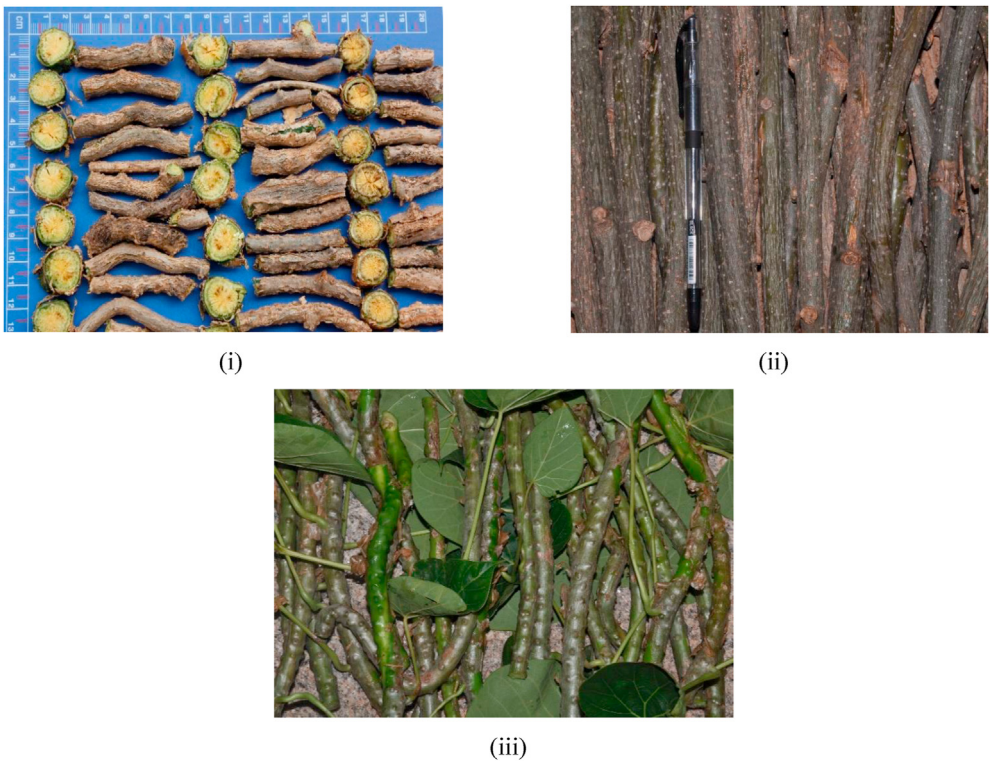

Fig. 4. Stems of i) *Tinospora cordifolia, ii) T. sinensis* and iii) *T. crispa*.

Table 2
Comparison of Pharmacological activities and Toxicology of three *Tinospora cordifolia, T. sinensis,* and *T. crispa*.

Pharmacological activity & Toxicology	*T. cordifolia*	*T. sinensis*	*T. crispa*
Antioxidant	Yes [37]	Yes [38]	Yes [27,39]
Anti-inflammatory	Yes [40–42]	Not well studied	Yes [43]
Anti-viral	Yes [29]	Yes [47]	Yes [46]
Immunomodulatory activity	Yes [45]	Yes [26]	Low [46]
Hepatoprotective activity	Yes [50,51]	Yes [52]	No. Chronic doses cause hepatotoxicity [53]
Toxicology	No [54,55]	NS	Yes [62,63]

NS-No Scientific publications available.

clearance, reduced inflammatory markers (hs-CRP, TNF α, and IL-6) and no side effects [36].

6.2. Antioxidant activity

The starchy material from the stems of TC is traditionally used for boosting immunity. Scientific studies on a polysaccharide (arabinogalactan) isolated from TC stems showed good antioxidant properties. The polysaccharide also demonstrated excellent reactivity towards DPPH, and superoxide radical scavenging activities [37]. *T. sinensis* was shown to have similar levels of antioxidant activity as TC [38]. Three chemicals isolated from *T. crispa*, namely N-cis-feruloyltyramine, N-transferuloyltyramine, and secoisolariciresinol, out-performed the synthetic antioxidant butylhydroxytoluene (BHT) [39]. Stems of *T. crispa* also possess antioxidant properties equivalent to ascorbic acid and BHT [27].

6.3. Anti-inflammatory and anti-pyretic activity

Carrageenan and histamine-induced rat edema was effectively treated with an aqueous extract of TC stem. TC methanolic extract mainly inhibited COX (Cyclooxygenase) and LOX (Lipooxygenase) enzymes [40]. TC stem extract provided a significant antipyretic effect on brewer's yeast-induced pyrexia model in an albino model when compared to standard anti-inflammatory and antipyretic drugs Diclofenac sodium and Paracetamol respectively [41]. TC extract and tinoporaside, a diterpene glycoside isolated from TC, have been shown to protect against inflammation-associated anemia, by modulating inflammatory cytokines *in vitro* and animal models [42]. The isolated protoberberine from TC was also found to inhibit the LOX enzyme. TC is therefore recognized as a medicinal herb with good anti-inflammatory properties and application in a wide range of inflammatory clinical conditions. *T. crispa* extract stabilized cellular membranes and reduced protein denaturation and anti-inflammatory activity at certain doses [43]. However, the anti-inflammatory or antipyretic effects of *T. sinensis* yet to be well explored.

6.4. Immunomodulatory activity

The research by the late Dr. Sharadini Dahanukar and team during the 90's on the immunomodulatory effect of TC is seminal, using *in vitro, ex vivo,* and animal models for various conditions [44]. A polysaccharide rich in glucose, fructose, and arabinose as monomer units is mainly responsible for TC's immunomodulatory activity. 11-hydroxymustakone, N-methyl-2-pyrrolidone, N-

formylannonain, cordifolioside A, magnoflorine, tinocordiside, and syringin are some of the other immunomodulatory active components found in this plant [45]. A double-blind placebo-controlled trial investigated the effect of TC on the formation of vaccination antibodies and GM−CSF (Granulocyte-macrophage colony-stimulating factor) levels. On the first day of hepatitis B immunization, TC was given at a dose of 1500 mg per day (for a matching placebo) and was continued for six months. Compared with the placebo group, TC treatment resulted in significantly greater anti-HBsAg titers after 3 months, which lasted until six months. The treated group also had significantly greater GM−CSF levels. This makes TC a potential vaccination adjunct [46].

T. sinensis prevents cyclophosphamide-induced anemia and has an immunomodulating effect [26]. Methanol, choloform, and n-butanol extracts of *T. crispa* have been shown to have no immune-enhancing effect; yet used as a treatment for various immunological illnesses such as autoimmune disease and malignancies. TC has a stronger immunomodulatory effect than *T. crispa* [46].

6.5. Antiviral activity

Tinosporin, a diterpenoid found in *T. cordifolia*, has antiviral properties that are particularly effective in the treatment of retroviruses and other viral disorders. Aqueous Extract from *T. cordifolia* has enhanced cytokine production and immune effector cell activation. Sanshamani Vati (also known as *Guduchi* ghana vati) is preventive and prophylactic medicine for COVID-19 that comprises an extract of TC. It is recommended to take 500 mg twice a day with warm water for 15 days [29]. *T. sinensis* hexane extract contains betulin, an antiviral agent. *T. sinensis* has antiviral properties, as evidenced by this research [47]. *Tinospora crispa* contains a flavonoid called apigenin, which is most recognized for its antiviral activities [46].

6.6. Hepatoprotective effects

Guduchi (TC) is traditionally recommended for treating *Kamala* (jaundice), reducing hepatosplenomegaly, and facilitating bile flow in obstructive lesions. It can also be administered as a single agent and is a component in more than a third of hepatoprotective formulas available on the Indian market [48]. It is a significant herb used to prevent hepatotoxicity [29], rather than cause hepatotoxicity. TC has been observed to maintain normal metabolism and to reduce the levels of specific enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin in carbon tetrachloride-induced liver damage. TC extract is an effective hepatoprotective agent, which may be due to several factors including antioxidant and free radical scavenging effects, as well as promotion of liver regeneration [45,49]. Patients with hepatic diseases who had evidence of fibrosis and/or immunosuppression were studied using a standardized aqueous extract. The most extensive clinical research was conducted in patients with obstructive jaundice, where the addition of TC (16 mg/kg/day) to conventional therapy before the surgical correction was found to significantly reduce mortality from 61.54% to 25% in patients with PTBD (Percutaneous transhepatic biliary drainage) and from 39% to 6.25% in patients without PTBD. This was linked to a reduction in the number of patients developing septicemia in the TC-treated group [50,51]. At a dose of 200 mg/kg, *T. sinensis* considerably reduced paracetamol-induced increased levels of serum ALT (Alanine transaminase), AST (aspartate aminotransferase), ALP (Alkaline phosphatase), and bilirubin, revealing normal liver architecture, indicating a hepatoprotective potential [52]. *T. crispa* however has the potential to cause hepatotoxicity [53].

6.7. Toxicology

Even at a high dose (9 g/kg body weight), leaves and stem of TC showed no adverse reactions or deaths in Swiss mice in an acute toxicity trial. There are no reports of highly toxic substances present in TC nor toxic or adverse reactions caused by TC [54−56]. In an acute toxicity investigation, Agarwal et al. (2002) found that a 3 g/kg dose of TC aqueous extract caused no adverse reaction and no death in the experimental rats [57]. In Human studies, *T. cordifolia* does not show any toxicological effects, even when tested at a very high dose (900 mg/day) in HIV patients [58] and patients with allergic rhinitis [59]. In an experimental study conducted by Adhvaryu et al., in 2008 to assess the efficacy of *Curcuma longa* (CL) and TC formulation to prevent anti-tuberculosis (TB) treatment (ATT) induced hepatotoxicity, it was found that when given as an adjunctive therapy to standard ATT to any type of TB patients, CL and TC substantially decreased the prevalence, and severity of hepatotoxicity [60]. TC was found to be safe at a dose of 500 mg/day for 21 days in healthy persons in a clinical trial with no significant adverse effects on the cardiovascular, renal, neurological, or gastrointestinal systems [61].

Acute toxicity analysis of the stem ethanol extract of *T. crispa* in animal studies demonstrated no negative effects or animal death at a dose of 4 g/kg. In a 6-month chronic toxicity investigation in experimental rats, hepatic and renal toxicities were reported when the *T. crispa* ethanol extract was given at a dose of 9.26 g/kg b. w. Overuse of *T. crispa* stems herbal preparation as a malaria prophylactic agent has been linked to hepatotoxicity in humans [62]. In a study by Cachet et al. (2018), a 57-year-old man was diagnosed with toxic hepatitis after consuming a *T. crispa* stem aqueous extract occasionally. *T. crispa*-induced hepatitis was confirmed by the absence of medical history, a clinical examination, and biochemical data. *T. crispa* has been linked to two earlier cases of acute hepatitis, both of which were treated with a different form of administration: prolonged use of the plant's tablets or pellets. A 37-year-old female was diagnosed with hepatitis after consuming *T. crispa* tablets (purchased in Indonesia) for ten weeks. The second case has included a 49-year-old male who had been taking *T. crispa* pellets (purchased from a Vietnamese market) orally for four weeks. Hepatitis was reversible in both patients within a few weeks.

In 2 of the 20 individuals treated for six months with a capsule version of *T. crispa* at a dosage of 1 g three times daily, significant elevations of liver enzymes were noted (that recovered to normal after withdrawing *T. crispa*). Similarly, 6 of the 36 patients who took 250 mg T. *crispa* dry powder capsule twice a day for two months had their Alanine aminotransferase **(**ALT) and aspartate transaminase (AST) levels elevated by more than three times their baseline levels in a double-blind, placebo-controlled experiment employing a crossover design. Chronic or frequent usage of *T. crispa* stem may result in toxic hepatitis, which is reversible after a few weeks without therapy [63]. There are no scientific studies on *T. sinensis* toxicity.

7. Discussion

This article reviews the Ayurvedic, phytochemical and pharmacological properties of TC. TC's use in COVID-19 is justified because of its antiviral, anti-oxidant, anti-inflammatory, and immune-boosting properties. TC is one of the most important herbs in Ayurveda, having a great potential (medicinal characteristics) for immune-modulation and treating a variety of diseases, and promoting well-being [10]. Moreover, TC has been scientifically proven to have a hepatoprotective effect and is used to treat Kamala (jaundice) and liver toxicity in various research investigations. It

has no reports of toxicity or health hazards before Nagral et al. and there have been no recorded side effects [56]. Therefore, Aabha Nagral's article "Herbal Immune Booster-Induced Liver Injury in the COVID-19 Pandemic-a case Series" published in June 2021, may have been due to the mistaken identity of TC and the use of *T. crispa* instead, which causes liver damage on chronic usage.

A major gap in Nagaral *et al.* study is the lack of vouched specimens and authentication of the botanical identity of the plant/plant products reported as consumed by the patients. While 4 of the six patients self-reported that they consumed the plant in the raw drug form (stem/powder), two of them reported use in the tablet and syrup form. Manufacturing/purchase details or morphological and phytochemical analysis of the raw drug/plant products could have helped further verify the authenticity of TC. However, these tests were not performed by the authors. While it has been mentioned as a limitation of the study in the paper, *T. cordifolia* (*Guduchi*) has become the dreaded 'suspect' without such analysis.

Another major lacuna in the Nagral study is the wide variation in the history of usage of TC across the six cases reported. What is specifically noteworthy is that case 4 (62-year-old female) had the onset of major symptoms two weeks before the visit to the doctor, but only had a history of consumption of 15 ml of syrup on alternate days for a month. Similarly, case 5 (56-year-old female) though symptom onset is not noted explicitly, only had a history of the boiled extract on 1 twig for 2–3 days per week for three weeks. The other four patients had above 3 months' consumption history. Clubbing cases 4 and 5 (especially with such short consumption history) with their unusually marked laboratory findings and significant hepatic changes, along with other four cases with long-term history, surely challenges the consistency of the observations in this case series. Except for case 1, the rest had significant comorbidities (two with type 2 diabetes; two with hypothyroidism, and one with thalassemia minor) and could have led to long-term consumption of concomitant medicines, for which detailed history and data are missing. These have already been highlighted in a communication published in clinical and experimental hepatology in response to the Nagral *et al.* study [64].

As seen in the current article, TC has substitutes and adulterants namely *T. sinensis* and *T. crispa* that are sold in the traded market as *Giloy* or *Guduchi* [18]. *T. crispa* has the potential to cause hepatotoxicity [53]. It's possible that instead of TC, a harmful botanical entity was used.

With the rise in demand for herbal remedies, the risk of malpractice and adulteration has also risen. The fall in faith in herbal medicine is due to the adulteration of herbal drugs. Unfortunately, most of the time, reported harmful effects of herbal preparations are attributable to the presence of inferior adulterant/substitutes rather than the original medicine [65]. Even though there are clear pharmacopeia standards for vouching for medicinal botanicals, quality control, and regulatory mechanisms could be better. Habitat loss and extinction of many species of plants lead to plant scarcity and are accountable for illegitimate substitution or adulteration. Therefore, environmental sustainability, forest protection, and improved agricultural methods are strategies to prevent adulteration.

8. Conclusion

A review of TC's Ayurvedic and pharmacological properties reveals no justifiable association between the drug-induced autoimmune-like hepatitis in the 6 patients observed by Nagral and consumption of TC. However, a robust pharmacovigilance system and consumer participatory database are the need of the hour to capture the adverse effects of Traditional Medicines, especially when it involves unsupervised self-administration of herbs from the market.

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Author contribution

Padma Venkatasubramanian: Conceptualization, Overall structuring of the MS, Scientific Review, Editing & Finalizing.

Keerthi Panneer Selvam: Data curation, Draft preparation & Final draft.

Unnikrishnan Payyappallimana: Validation & editing of Ayurvedic aspects.

Kaliamoorthy Ravikumar: Validation & editing of Botanical aspects.

Declaration of Competing Interest

None.

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