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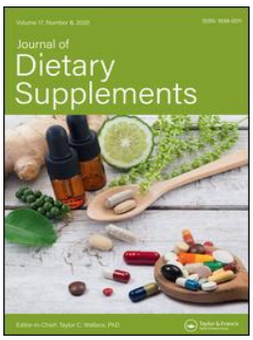

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REVIEW

Tinospora Cordifolia: A review of its immunomodulatory properties

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ABSTRACT

Emergent health threats have heightened human awareness of the need for health and wellness measures that promote resilience to disease. In addition to proper nutrition and exercise, health-conscious consumers are seeking natural-based modalities, *e.g.* botanical preparations, that positively impact the immune system. In Ayurvedic ethnomedicine, *Tinospora cordifolia (T. cordifolia*), a deciduous climbing shrub indigenous to India, has been used to historically to combat acute and chronic inflammation as well as to promote a balanced immune response. As a dietary supplement, *T. cordifolia* has been administered most often as a decoction either alone or in compositions containing other medicinal plant extracts of the *Terminalia* and *Phyllanthus* species. Extensive phytochemical characterization of aqueous and alcoholic extracts of different *Tinospora* species has identified over two hundred different phytochemicals from non-overlapping chemical classes with the most abundant being diterpenoids containing the clerodane-type skeleton. Numerous pharmacology studies have demonstrated that *T. cordifolia* modulates key signaling pathways related to cell proliferation, inflammation, and immunomodulation. However, rigorous dereplication studies to identify active constituents in various *T. cordifolia* extracts and their fractions are lacking. In this review, we will summarize the current information regarding *T. cordifolia*'s ethnomedicinal uses, phytochemistry, pharmacological activities, and safety in order to highlight its potential as an immunomodulatory dietary supplement.

KEYWORDS

Dietary supplement; nutraceutical; extract; *Tinospora cordifolia*; immune health

Introduction

As the incidence of and susceptibility to acute and chronic diseases continuously increases, many health-conscious individuals have shifted their mindset from "treatment-centric", *viz.*, a primary reliance on pharmaceutical interventions, to one that incorporates measures and routines that promote disease prevention and resilience. For example, recent emergent health threats have heightened human awareness of the importance of and need for natural-based modalities that improve health and wellness. In this context, potential candidate botanical preparations are those that both promote a

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vigorous, well-regulated immune response and mitigate co-morbidities (*e.g.* diabetes) that weaken the immune system and pre-dispose to either bacterial or viral infection. The ethnomedicine literature including traditional Chinese and Ayurvedic medicine is rife with preclinical and clinical substantiation data that support the immunomodulatory-related structure function claims of herbal preparations derived from numerous well-known medicinal plants such as *Echinacea, Curcuma, Camellia,* etc. The increased emphasis on human disease prevention and resilience has provided the impetus to explore and highlight additional, lesser-known medicinal plants with purported anti-inflammatory and immunomodulatory activity.

The genus *Tinospora* (Menispermaceae), which comprises 34 different species, is a large, glabrous, perennial, deciduous, climbing shrub of weak and fleshy stem found throughout Asia, Africa, and Australia. A prominent member of the *Tinospora* genus, *T. cordifolia* (Willd.) Hook. f. and Thoms (*Guduchi*), is found throughout the tropical Indian subcontinent and China and has numerous ethnomedicinal applications in Ayurvedic pharmacology (Singh and Saxena 2017; Upadhyay et al. 2010). *T. cordifolia* is commonly referred to as Guduchi, which is Sanskrit for "one which protects the entire body". It is a rich natural source of zinc and copper–trace elements with antioxidant activity. In Ayurveda, *T. cordifolia* has been used traditionally as a decoction, alone or in combination with other rasayana herbs that possesses adaptogenic properties (e.g. *Terminalia* and *Emblica*), to enhance energy levels, immunity, general health and longevity (Rege et al. 1999). In this focused review, we will detail *T. cordifolia*'s ethnomedicinal uses, phytochemistry, pharmacological activities, and safety with an emphasis on immunity and infection.

Phytochemistry

Extensive phytochemical characterization of *Tinospora* species has identified over two hundred different phytochemicals from diverse chemical classes (reviewed in (Chi et al. 2016)) with diterpenoids representing the most abundant chemical class. For example, *T. cordifolia* contains diterpenoids, *e.g.* cordifolides (Pan et al. 2012), with the clerodyne-type skeleton: Type 1 (C-8/C-12 linking lactone ring present) and Type 2 (C-8/C-12 linking lactone ring absent). However, *T. cordifolia* does not appear to contain additional structurally related diterpenes found in other members of the *Tinospora* genus (e.g. *T. rumphii*, *T. crispa, and T. baenzigeri*). Tinosponone and tinocordioside, which are cyclobutene ring-containing tricyclic terpenes, are found in *T. cordifolia*. In addition, *T. cordifolia* was found to contain the daucane-type sesquiterpenes tinocordifolioside (Maurya et al. 1997; Maurya and Handa 1998), tinocordifolioside acetate (Maurya and Handa 1998), and tinocordifilin (Maurya and Handa 1998), as well as the monoterpenoid angelicoidenol-2-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (Phan et al. 2010).

Alkaloids represent another abundant class of phytoconstituents in the *Tinospora* genus. These include alkaloids of the berberine class (berberine (Mohan et al. 2017; Maurya et al. 1995; Srinivasan et al. 2008; Palmieri et al. 2019), palmatine (Patel and Mishra 2012; Bisset and Nwaiwu 1983) reticuline (Bala et al. 2015), and jatrorrhizine (Patel and Mishra 2012; Bala et al. 2015) as well as the aporphine class (magnoflorine

Table 1. Tinospora cordifolia phytochemistry and pharmacology.

Phytoactive	Class	Plant Part	Activity
Cordifolioside	Phenylpropanoid Glycoside	Stem	↑ phagocytosis (Maurya et al. 1996; Kapil and Sharma 1997; Sharma et al. 2012)
Syringin	Phenylpropanoid Glycoside	Stem	↑ phagocytosis (Kapil and Sharma 1997; Sharma et al. 2012)
Cordioside	Clerodane furano diterpene	Stem	↑phagocytosis (Kapil and Sharma 1997)
Magnoflorine	Benzylisoquinoline alkaloid	Stem, Root	↑phagocytosis; ↑ROS (Sharma et al. 2012)
N-Formylannonain	Benzylisoquinoline alkaloid	Stem, Root	↑phagocytosis; ↑ROS (Sharma et al. 2012; Bala et al. 2015)
Tinocordiside	Cadinane sesquiterpene	Stem	↑phagocytosis; ↑ROS (Sharma et al. 2012)
11-hydroxymuskatone	Cadinane sesquiterpene	Stem	↑phagocytosis; ↑ROS (Bala et al. 2015)
G1-4A	Arabinogalactan polysaccharide	Stem	↓mortality (LPS septicemia model) (Desai et al. 2007); ↑Th1, ↓Th2 cytokines (Gupta et al. 2016); ↑macrophage activation; ↑mitogenesis (Chintalwar et al. 1999)
RR1	α-D-glucan polysaccharide	Aerial Parts	↑phagocytosis (Nair et al. 2006); ↑Th1 cytokines (Nair et al. 2004)
Guduchi immunomodulatory protein (ImP)	Protein	Stem	↑phagocytosis; ↑mitogenesis; ↑bactericidal (Aranha et al. 2012)

(Maurya et al. 1995; Patel and Mishra 2012; Bala et al. 2015), menisperine (Maurya et al. 1995; Bala et al. 2015), tinoscorside A[8], and tinoscorside B[8]. Other chemical classes identified in *T. cordifolia* include steroids (derivatives of *β*-sitosterol (Maurya et al. 1995; Bala et al. 2015)) and polysaccharides including glucose, arabinose, xylose, galactose, rhamnose, and mannose (Jahfar and Azadi 2004; Sharma et al. 2010). A more comprehensive list of phytochemicals identified in *T. cordifolia* and plant parts from which they were isolated can be found in Table 1.

T. cordifolia extracts

The pharmacologic activity of *T. cordifolia* has often been investigated using either non-aqueous or aqueous-based decoctions of aerial parts–primarily leaf or stem. For example, *ex vivo* application of extracts of *T. cordifolia* leaf (methanolic) and stem (ethyl acetate) to male Wistar albino rat liver homogenates subjected to peroxyl radical induced oxidative stress were found to have the greatest anti-oxidant activity (Ilaiyaraja and Khanum 2011). Moreover, intraperitoneal administration of hexane fractions of *T. cordifolia* dose-dependently inhibited tumor growth and induced apoptosis in Ehrlich ascites tumor bearing Swiss Albino female mice (Thippeswamy and Salimath 2007). Ethanolic fractions have also been used to demonstrate *T. cordifolia*'s antimicrobial activity against *S. aureus* and *K. pneumoniae* (Bonvicini et al. 2014) as well as its neuro-protective potential as manifested by increased dopamine levels and mitochondrial complex I activity (Kosaraju et al. 2014). *T. cordifolia*'s CNS activity was further illustrated

when an orally-administered ethanolic extract composition comprised of equal portions of *T. cordifolia*, *Bacopa monnieri*, and *Evolvulus alsinoides* elicited synergistic nootropic effects in a Wistar male rat model of scopolamine-induced amnesia (Gupta et al. 2013). Interestingly, ethanolic and methanolic *T. cordifolia* fruit extracts demonstrate measurable antioxidant activity (methanolic greater than ethanolic), but still less than leaf or stem extracts (Ilaiyaraja and Khanum 2011; Khan 2011). Oral administration of ethanolic extracts have also been shown to exert hepatoprotective effects in CCL4 mouse model (Kavitha et al. 2011).

Aqueous *T. cordifolia* extracts have also demonstrated promising pharmacologic activity in preclinical substantiation studies. For example, oral administration of freeze-dried aqueous fractions of *T. cordifolia* stem (40 mg/kg) enhanced peritoneal macrophage activity, which was associated with enhanced nonspecific immune responses, in a murine model of carbon tetrachloride-induced hepatotoxicity (Sengupta et al. 2011). In addition, oral administration of aqueous extracts of *T. cordifolia* exerted antioxidant and hepatoprotective activity in a murine model of paracetamol-induced hepatotoxicity (Kaushik et al. 2017). Lastly, reduced proliferation and induced differentiation were observed in C6 glioma cell treated with aqueous ethanolic ingredients of *T. cordifolia* (Mishra and Kaur 2013). It is important to note that most commercial preparations of *T. cordifolia* represent stem water extracts (10-15:1) assayed for total bitters (bitter principles such as tinosporine not less than 5.00%) and total polysaccharides (not less than 20%).

Mechanism(s) of action

Dereplication studies aimed at identifying phytochemical actives in *T. cordifolia* extracts and fractions have yielded important insight into its immunomodulatory mechanism(s). Initial studies with *T. cordifolia* found that the glycosides cordifoliosides A and B isolated from the stem possess immunopotentiating activity as evidenced by increased IgG antibody production in Balb/c mice subcutaneously injected with sheep red blood cells (Maurya et al. 1996). Using the same murine model, enhanced antibody production was observed for cordifolioside A and other *T. cordifolia* active principles including syringin, cordiol, and cordioside (Kapil and Sharma 1997). Kapil *et al.* also observed that cordifolioside A, cordioside, and cordiol increased peritoneal macrophage phagocytic activity (Kapil and Sharma 1997).

The aforementioned studies prompted other groups to use dereplication strategies to identify individual phytochemicals responsible for *T. cordifolia*'s immunomodulatory activity. For example, Sharma *et al.* extracted *T. cordifolia* stem powder using either hot water or methanol: water followed by fractionation with n-hexane, ethyl acetate, chloroform, n-butanol, and water (Sharma et al. 2012). The ethyl acetate, chloroform, and water fractions, as well as the hot water extract, displayed immunomodulatory activity as demonstrated by enhanced neutrophil phagocytosis. Dereplication studies identified five known phytochemicals (N-formylannonain, cordiofolioside A, magnoflorine, tinocordiside, and syringin) and two (11-hydroxymuskatone and N-methyl-2-pyrrolidone) that were previously undescribed in a natural source. Treatment of human neutrophils with magnoflorine, tinocordiside, 11-hydroxymuskatone, and N-methyl-2-pyrrolidone

increased phagocytosis and release of reactive oxygen species–biomarkers of enhanced PMN activity. Bala *et al.* extracted *T. cordifolia* stem powder using ethanol: water followed by fractionation with n-hexane, ethyl acetate, n-butanol, and water (Bala et al. 2015). Using the murine splenocyte proliferation assay as a proxy for immunomodulatory activity, they showed that the water extract was the most potent fraction and that purified N-formylannonain and 11-hydroxymuskatone were responsible for the immunoenhancing effects of *T. cordifolia*.

Numerous polysaccharides with immunomodulatory activity have been identified as phytochemical components of *T. cordifolia* (Jahfar and Azadi 2004; Venkata Rao and Venkateswara Rao 1981; Nair et al. 2004; Chintalwar et al. 1999; Roja et al. 2005; Jahfar 2003). For example, Chintalwar *et al.* first identified the arabinogalactan polysaccharide G1-4A in water extracts of the *T. cordifolia* stem and demonstrated its polygenic mitogenic activity in B-cells (Chintalwar et al. 1999). In a subsequent study, pretreatment with G1-4A prevented lipopolysaccharide (LPS)-induced mortality in a murine model of septicemia (Desai et al. 2007) where reduced mortality was associated with a blunted tumor necrosis factor-alpha (TNF-α) response, increased circulating TNF receptor levels, and decreased nitric oxide release by splenic adherent cells. The ability of G1-4A to mitigate host immune responses has also been demonstrated in a BALB/c murine model of drug-resistant *Mycobacterium tuberculosis* (Gupta et al. 2016). G1-4A treatment reduced pulmonary bacillary burden, which correlated with an increased Th1 cytokine and a decreased Th2 cytokine profile. Pretreatment of murine RAW264.7 macrophages significantly induced surface expression of major histocompatibility complex-II (MHC-II) and CD-86, which are markers of classically activated macrophages (M1). M1 macrophages, in general, exhibit microbicidal activity characterized by increased elaboration of pro-inflammatory cytokines and nitric oxide.

The aforementioned studies led investigators to examine B-cells and macrophages as potential target cell populations for G1-4A. For example, fluorescence microscopy studies identified B-cells (minor) and macrophages (major) as targets of G1-4A, which led the authors to speculate G1-4A and LPS shared the same cellular target (Desai et al. 2007). This supposition was confirmed by Raghu *et al.* who used anti-TLR4 (toll-like receptor 4) antibodies to demonstrate that G1-4A acted as a TLR4 agonist and stimulated murine B-cells leading to increased lymphocyte proliferation and splenic cellularity (Raghu et al. 2009). Previous reports of G1-4A's ability to activate murine macrophages, as evidenced by increased phagocytosis, were confirmed and found to be dependent upon ERK and NF-κB. In later studies, Gupta *et al.* also found that G1-4A elicited a TLR4-MyD88 dependent Th1 cytokine response characterized by up-regulation of TNF-α and IL-1β and an M1 phenotype highlighted by increased MHC-II and CD-86 surface expression in murine macrophages (Gupta et al. 2017). Pharmacologic inhibitors demonstrated the role of key cell signaling pathways, including p38, ERK, and JNK MAPKs, in macrophage activation by G1-4A.

Beta glucans (β-glucans) are another class of polysaccharides with immunostimulatory properties (Brown and Gordon 2003). β-glucans interact with their cognate receptors on macrophages (e.g. CD11b, TLR2, TLR6, etc.) to stimulate a Th1 cytokine response (Gantner et al. 2003). It was previously thought that the β-glycosidic linkage found in β-glucans was *sine quo non* for immune enhancing activity. However, recent

studies demonstrated that α linkages found in α-glucans could also impart immunostimulatory activity (Bao et al. 2002; Wang et al. 2003). For example, Nair *et al.* previously isolated a novel α-glucan, (1,4)-α-D-glucan (RR1), from *T. cordifolia* and found that it potently stimulated a Th1 cytokine response in natural killer, T, and B cells (Nair et al. 2004). Mechanistic studies with RR1 revealed that RR1 stimulated phagocytic activity of RAW264.7 macrophages that was independent of CD11b surface expression (Nair et al. 2006). Additionally, it was found that RR1, unlike G1-4A, exerted immunostimulatory activity by acting as a TLR6 agonist in HEK293 cells.

T. cordifolia extracts also contain immunomodulatory activity independent of the aforementioned low molecular weight phytochemicals and polysaccharides. For example, an immunostimulatory protein, known as guduchi immunomodulatory protein (ImP), was found to be present in dry as well as fresh *T. cordifolia* stem powder (Aranha et al. 2012). However, it was essentially absent in *T. cordifolia* leaf extracts. The guduchi ImP demonstrated mitogenic activity on murine splenocytes and thymocytes. Moreover, guduchi ImP stimulated murine macrophage phagocytic and bactericidal activity without demonstrating hemagglutination activity.

Safety/toxicity

The safety profile of orally administered *T. cordifolia* has been evaluated in both healthy individuals as well as in individuals with various diseases (summarized in Table 2). No adverse effects have been reported following chronic, *i.e.* up to eight weeks, administration of *T. cordifolia* aqueous stem extracts in doses up to ~1 gram/day. In addition, the potential for *T. cordifolia* to elicit herb-drug interactions *via* cytochrome P-450 (CYP450) modulation has also been evaluated *in vitro*. An investigation of *T. cordifolia* metabolism in pooled rat liver microsomes and recombinant human CYP450 enzymes revealed that hydroalcoholic *T. cordifolia* extract weakly inhibited several CYP450 drug metabolizing enzymes including CYP3A4 (IC_{50} = 127 μg/mL), CYP2D6 (IC_{50} = 138 μg/mL), CYP2C9 (IC_{50} = 110 μg/mL), and CYP1A2 (IC_{50} = 127 μg/mL) (Bahadur et al. 2016). Similar weak inhibition by was noted by Sahu *et al* for CYP3A4 (IC_{50} = 3.44 mg/mL), CYP2D6 (IC_{50} = 170 μg/mL), CYP2C9 (IC_{50} = 92 μg/mL), CYP2C19

Table 2. Tinospora cordifolia clinical trial data.

Population (n)	Oral Dosage Regimen	Form (source)	Adverse Events
Obstructive jaundice ($n = 15$)	16 mg/kg x1 daily for 5 weeks	Aqueous Extract (Stem)	No adverse events reported (Rege et al. 1993)
Healthy volunteers ($n = 15$)	500 mg x1 daily for 3 weeks	Aqueous Extract	No adverse events reported (Bairy et al. 2004)
Allergic rhinitis ($n = 37$)	300 mg x3 daily for 8 weeks	Aqueous Extract (Stem)	No adverse events reported (Badar et al. 2005)
Diabetic foot ulcer ($n = 23$)	Regimen not reported; treatment for 4 weeks	Aqueous Extract (Stem)	No adverse events reported (Purandare and Supe 2007)
Healthy volunteers ($n = 15$)	500 mg x1 daily for 3 weeks	Aqueous Extract (not reported)	No adverse events reported (Karkal and Bairy 2007)
Healthy volunteers ($n = 10$/ treatment group)	150 or 300 mg x1 daily for 4 weeks	Aqueous Extract (Stem)	No adverse events reported (Salve et al. 2015)
Type 2 diabetes ($n = 20$)	1000 mg x three daily for 24 weeks		Elevation of liver enzymes in two subjects (Sangsuwan et al. 2004)

(IC_{50} = 866 μg/mL), and CYP1A2 (IC_{50} = 1.49 mg/mL) (Sahu et al. 2018). Based on these data, it would be prudent to monitor herb-drug interaction potential in patients consuming *T. cordifolia* and prescription medications that rely on one or more of the aforementioned CYP450 drug metabolizing enzymes as a primary mechanism of hepatic elimination.

Several studies have been conducted in animals to evaluate the toxicity of *T. cordifolia*. In an acute toxicity study, male Swiss albino mice and Swiss albino rats were orally administered a single dose (in 0.5 mL or 1 mL increments) of methanolic or aqueous extract of *T. cordifolia* up to 3500 mg/kg body weight. Upon observation at 24 h, no deaths or other signs of toxicity were observed (Agarwal et al. 2002). In a second acute toxicity study, Swiss albino mice received either water or ethanolic extracts (100 mg/kg orally) for 10 days (Manjrekar et al. 2000). No change in hemoglobin levels were noted, however, there was an increase in total white blood cell count in treated mice.

The findings from a series of genetic toxicity assays conducted by Chandrasekaran et al. (2009) suggest that *T. cordifolia* aqueous extract is of low concern for mutagenicity and clastogenicity (Chandrasekaran et al. 2009). A bacterial reverse mutation assay with five standard strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, and TA1535) conducted with or without metabolic activation was negative at concentrations up to 5000 μg/plate. Likewise, an *in vitro* chromosomal aberration test in human peripheral blood lymphocytes with or without metabolic activation was also negative at concentrations up to 3000 μg/mL. Finally, an *in vivo* assay of clastogenicity was conducted in male Balb/c mice that were orally administered 150, 200 or 250 mg/kg-day *T. cordifolia* for seven days. A subsequent micronucleus and Comet assay assessment of bone marrow erythrocytes and peripheral blood lymphocytes revealed no remarkable findings related to clastogenicity or DNA damage (Chandrasekaran et al. 2009).

Immune modulation

Polymorphonuclear leukocytes (PMNs) are immune cells that possess small granules filled with enzymes (e.g. myeloperoxidase and lysozyme) and molecules (e.g. superoxide and histamine) that are released during infections, allergic reactions, and asthma. The PMN family includes neutrophils, eosinophils, mast cells, and basophils. In addition to their secretory defense mechanisms, neutrophils and mast cells protect the body by phagocytizing ("devouring") foreign particles and bacteria. Monocytes and macrophages, which represent agranulocytic leukocytes, also possess phagocytic activity. Together with neutrophils, they comprise the professional phagocytes, which are characterized by the expression of receptors that sense foreign bodies such as bacteria. Due to its immunomodulatory activity, *T. cordifolia* has been extensively investigated in preclinical animal models and human diseases related to inflammation and infection.

Allergic rhinitis

Allergic rhinitis involves type 2 helper (Th2) cell driven mucosal inflammation caused by IgE-mediated reactions to inhaled allergens (Bousquet et al. 2008). Allergic rhinitis symptoms include sneezing, nasal pruritis, airway obstruction, and clear nasal discharge.

It is estimated that up to 40% of people with allergic rhinitis have or will have asthma (Shaaban et al. 2008). Due to *T. cordifolia*'s anti-inflammatory and anti-allergic properties, Badar *et al.* conducted a randomized double-blind placebo-controlled trial to assess the efficacy of *T. cordifolia* extract in individuals with allergic rhinitis (Badar et al. 2005). Patients ($n = 75$) were randomized to receive either placebo or an aqueous extract of *T. cordifolia* stem (300 mg/day) orally for eight weeks. After eight weeks, subjects receiving *T. cordifolia* extract reported experiencing less sneezing, nasal discharge, nasal obstruction and itching compared to subjects in the placebo arm. In addition, *T. cordifolia* treated subjects were found to have reduced eosinophil and neutrophil counts, as well as absent goblet cells, in their nasal smears. *T. cordifolia* was well-tolerated and leukocyte numbers and cytology results correlated with clinical findings implying that *T. cordifolia* may serve as a natural remedy for sufferers of allergic rhinitis.

Sepsis

Sepsis is caused by a dysregulated infection response characterized by the release of excessive pro-inflammatory cytokines, a condition termed the systemic inflammatory response syndrome (SIRS) or "cytokine storm". There are approximately 1.7 million sepsis cases per year with nearly 270,000 resulting in death (Centers for Disease Control and Prevention [Internet]. 2016). One of the first studies to examine the impact of *T. cordifolia* on SIRS found that oral administration of *T. cordifolia* (100 mg/kg for 15 days before and five days after surgery) improved mortality and bacterial clearance in the rat cecal ligation and puncture model of sepsis (Dahanukar et al. 1988). In addition, *T. cordifolia* decreased mortality in cyclophosphamide-induced immunosuppressed mice challenged with common pathogens *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, or *Klebsiella pneumonia* (Thattet and Dahanukar 1989). Subsequent studies demonstrated that *T. cordifolia* extract reduced mortality in a murine *E. coli*-induced peritonitis model similar to the antibiotic gentamicin (Thatte et al. 1992). *T. cordifolia* treatment was associated with improved bacterial clearance as well as phagocytic and bactericidal activity of neutrophils.

Immunosuppression associated with deranged hepatic function and sepsis results in poor surgical outcome in extrahepatic obstructive jaundice. Rege *et al.* determined that in humans with obstructive jaundice, as well as in a rat model of cholestasis, phagocytic and microbiocidal activity of polymorphonuclear (PMN) cells was depressed, which predisposed to infection (Rege et al. 1989). Administration of a water extract of *T. cordifolia* (100 mg/kg for 7 days post-initiation of cholestasis in rats) resulted in improved phagocytic and microbiocidal activity as well as reduced mortality following *E. coli* infection. The ability of *T. cordifolia* to improve immune-mediated responses in human and animal models of immunosuppression suggested *T. cordifolia* may exert immunostimulatory activity by bolstering host defense mechanisms.

In an effort to further extend findings in animal models of infection to humans, a randomized clinical study was conducted to evaluate the effect of *T. cordifolia* on surgical outcome in patients with malignant obstructive jaundice (Rege et al. 1993). Thirty patients were randomly divided into two groups, matched with respect to clinical features, impairment of hepatic function (as judged by liver function tests including

antipyrine elimination) and immunosuppression (phagocytic and killing capacities of neutrophils). During the period of biliary drainage, Group I received conventional management (*i.e.* vitamin K, antibiotics and biliary drainage) while Group II received conventional management plus dried aqueous stem extracts of *T. cordifolia* (16 mg/kg/day orally in three divided doses). No difference in hepatic function was noted between the two groups, however, the phagocytic and killing capacities of neutrophils was strengthened in patients receiving *T. cordifolia*. Moreover, septicemia was noted in half of the Group I patients as opposed to none in Group II, notwithstanding the fact that post-drainage bactobilia did not differ between the groups. Importantly, survival was doubled in Group II, which received *T. cordifolia*, compared to Group I (92.4 vs 40%, $p < 0.01$). Together, the aforementioned studies suggest that *T. cordifolia* may be beneficial in patients with bacteremia due to its anti-bacterial and immune-enhancing activity.

Salmonella Typhi infection

Salmonella enterica serotype Typhi (*S.* Typhi) is a gram-negative bacteria that causes typhoid fever in approximately 300 Americans every year. In a recent investigation, Alsuhaibani *et al.* examined the activity of aqueous and methanolic extracts of *T. cordifolia* (AETC and METC) against *S.* Typhi (Alsuhaibani and Khan 2017). In both the broth dilution and agar well diffusion assays, AETC and METC demonstrated anti-Salmonella activity at minimum inhibitory concentrations of 64 μg/mL and 32 μg/mL, respectively. Moreover, the immune-stimulating activity of both AETC or METC was demonstrated as evidenced by enhanced secretion of proinflammatory cytokines such as TNF-α and IL-1β by *S.* Typhi exposed J774 murine macrophages. Lastly, while both METC and AETC improved bacterial clearance and survival in *S.* Typhi-infected Balb/C mice, METC demonstrated more potent activity than AETC. Enhanced bacterial clearance and associated survival coupled with T. cordifolia extract's anti-bacterial activity extend previous results demonstrating the potential therapeutic value of *T. cordifolia* in conditions associated with bacteremia.

Toxoplasmosis

Toxoplasmosis is a wide-spread zoonotic infection that, due to poor infection control and lack of effective medications, remains endemic to many parts of the world. In an effort to identify potential botanical-based interventions for toxoplasmosis, Sharif *et al.* examined the activity of an ethanolic stem extract of *T. cordifolia* against *Toxoplasma gondii*, the organism responsible for toxoplasmosis (Sharif et al. 2019). Using Vero cells derived from kidney epithelium, *T. cordifolia* ethanolic extracts, veratrine (primarily a mixture of the alkaloids cevadine and veratridine present in *Veratrum* plants), and clindamycin were compared with regard to activity against host cell invasion and intracellular replication of *T. gondii*. Dose dependent (1.56 to 200 μg/mL) studies demonstrated that *T. cordifolia* possessed equal or greater anti-toxoplasma activity and exhibited a superior selectivity index, as determined by comparing activity to cytotoxicity, relative to either veratrine or clindamycin. Moreover, *T. cordifolia* treatment led to a reduction in both infection index (70%) and intracellular proliferation (80%). Together these data

suggest that ethanolic *T. cordifolia* stem extracts represent a potential safe and effective modality for fighting toxoplasmosis.

Diabetic wounds

As a consequence of their impaired immune status, diabetic patients are at significant risk of developing foot ulcers defined as chronic non-healing wounds associated with skin disruptions. Purandare *et al.* evaluated aqueous *T. cordifolia* stem extracts (no dosage provided) as an adjuvant therapy for diabetic foot ulcers (Purandare and Supe 2007). Briefly, a prospective, double-blind, randomized placebo-controlled study was conducted in diabetics ($n = 45$) receiving daily administration of *T. cordifolia.* Subjects were randomized to receive either *T. cordifolia* ($n = 23$, mean ulcer duration = 21.1 days) or placebo ($n = 22$, mean ulcer duration = 30.4 days). At baseline, ulcers were classified by wound morphology and severity according to the Wound Severity Score (Pecoraro-Reiber system) along with measurement of mean ulcer area, depth, and perimeter. Blood was obtained for assessment of PMN phagocytosis. At four weeks, subjects receiving *T. cordifolia* experienced fewer debridements whereas other parameters used to assess treatment response were not different between treated and control subjects. At three months, PMN phagocytosis was significantly improved in *T. cordifolia* treated subjects compared to controls (3.9 vs. 2.3; $p = 0.048$). Based on its immunostimulatory properties, *viz.*, fewer debridements and enhanced PMN phagocytosis in treated subjects, the authors concluded that *T. cordifolia* may be viewed as a potential adjuvant in preventing chronic diabetic foot ulcers.

Conclusions and future directions

In this review, we have highlighted the immunomodulatory properties of *T. cordifolia* while acknowledging that *T. cordifolia* extracts have demonstrated activity in a wide-range of chronic inflammatory disorders such as diabetes (Thomas et al. 2016) and cognitive decline (Sharma et al. 2020). Immunomodulatory activity is mediated by a variety of phytochemicals from diverse chemical classes including polysaccharides, alkaloids, cadinane sesquiterpenes, and phenylpropanoid glycosides that are found the aerial parts of *T. cordifolia* contain a (Table 1). Representatives from each of the aforementioned chemical classes variously promote PMN phagocytosis and alter the balance of Th1 and Th2 cytokines. Pharmacologic activity is mediated *via* diverse signaling networks, *e.g.* TLR4, that leads to regulation of the balance between Th1 and Th2 cytokines; a key feature, considering dysregulation of the host Th1/Th2 cytokine profile is at the heart of the so-called "cytokine storm" (Figure 1). The vast majority of pre-clinical and clinical studies involving *T. cordifolia* suggest it is safe at the doses ingested and that *T. cordifolia* poses minimal risk with regard to herb-drug interaction potential.

Emergent health threats have heightened human awareness of the importance of and need for natural-based modalities that improve health and wellness. In this context, potential candidate botanical preparations are those that both promote a vigorous, well-regulated immune response and mitigate co-morbidities (*e.g.* diabetes) that weaken the immune system and pre-dispose to either bacterial or viral infection. The vast majority of

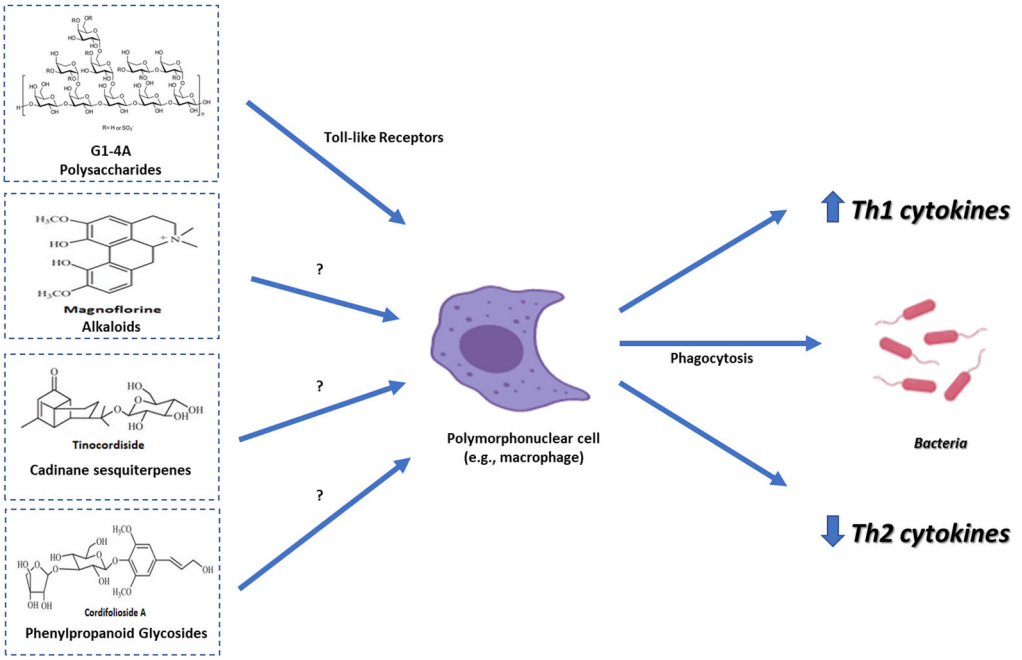

Figure 1. *Tinospora cordifolia* mechanism(s) of action. *T. cordifolia* extracts contain numerous active phytochemicals from a variety of classes including polysaccharides (*e.g.* G1-4A), alkaloids (*e.g.* magnoflorine), cadinene sesquiterpenes (*e.g.* tinocordiside), and phenylpropanoid glycosides (*e.g.* cordifolioside A). In general, *T. cordifolia* extracts exert immunomodulatory activity by promoting polymorphonuclear cell (*e.g.* macrophage) phagocytosis of bacteria and as well as by helping to maintain an appropriate balance between Th1 cytokines, known to produce pro-inflammatory responses that promote killing of intracellular pathogens, and Th2 cytokines, which, in excess, can counteract Th1-mediated microbicidal activity. Polysaccharides like G1-4A are posited to act through toll-like receptors, whereas the cellular target for members of the other phytochemical classes remains unknown.

preclinical and clinical substantiation data support the notion that *T. cordifolia* augments the host defense response to bacterial infection. However, caution should be exercised when extrapolating said activity to host viral responses since data to support such structure function claims are lacking. Consequently, future dereplication studies aimed at determining which, if any, additional phytochemicals mediate immunomodulatory activity as well as substantiation studies designed to explore *T. cordifolia*'s impact on the host's anti-viral response are needed. In summary, *T. cordifolia* represents a potential botanical option for augmenting the body's natural defense mechanisms, particularly against bacterial threats, based upon a wealth of immunomodulatory preclinical and clinical substantiation data.

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