Comparative immunomodulation potential of *Tinospora cordifolia* (Willd.) Miers ex Hook. F., *Tinospora sinensis* (Lour.) Merrill and *Tinospora cordifolia* growing on *Azadirachta indica* A. Juss.

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Guduchi has been widely used in the traditional medicine as an immunomodulator. Description of guduchi in Ayurvedic literature resemble with *T. sinensis* rather than with commonly available *T. cordifolia* and hence this may be used as substitutes for *T. sinensis*. *T. cordifolia* growing on *Azadirachta indica* commonly called Neem-guduchi has more immunomodulatory potential. Thus, immunomodulatory activity of three *Tinospora* spp. was assessed by checking humoral and cell mediated immune responses to the antigenic challenges with sheep RBCs and by neutrophil adhesion tests on albino Wistar rats using Guduchi-Satwa, a well known dosage form. Results revealed that Neem-guduchi possesses higher immunomodulatory potential at the dose of 300 mg/kg, po and validated the traditional claim. Hence, Neem-Guduchi can be employed in immunomodulatory formulation prepared using guduchi.

**Keywords:** Guduchi Satwa, Immunomodulation, Neem-guduchi, *Tinospora cordifolia, Tinospora sinensis*

Guduchi is often employed by Ayurvedic practitioners to boost patient’s immune system. In Indian sub-continent, genus *Tinospora* (family Menispermaceae) occurs in four different species, *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms, *Tinospora sinensis* (Lour.) Merr., *Tinospora crispa* (L.) Miers ex Hook. f. & Thoms and *Tinospora glabra* (Burm f.) Merrill. The *Tinospora* plants are locally known as Amrita, Amritavalli, Chinnobhava, Chakralakshana, Guduchi, Gulvel, Gurch, Kaduvel, Kundalini, Madhuparni, Sudarsana Tantrika, Vatsadan etc.  

*T. cordifolia* is a large, glabrous, perennial, deciduous, climber and widely used in folk and Ayurvedic system of medicine. T. *cordifolia* has been used in Ayurvedic ‘Rasayana’ as immunomodulator. *T. cordifolia* is one of the major constituent of several Ayurvedic preparations used preferably for general debility, dyspepsia, fever and urinary diseases. The mature stem of *T. sinensis* has been used to treat fever, jaundice and burning sensation. In China, the fresh leaves and stem is used in the treatment of chronic rheumatism and to treat piles and ulcerated wounds. The scientific validation studies on *T. sinensis* reported to possess anti-inflammatory and anti-diabetic activities.

Immune compromised function is involved in the etiology as well as pathophysiology of many diseases. Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of ‘Rasayana’ in Ayurveda is based on related principles. Herbal drugs are known to possess excellent immunomodulatory properties and generally act by stimulating both specific and non specific immune response. Many plants used in traditional medicine have immunomodulatory activity. Some of these medicines stimulate both humoral and cell-mediated immunity, while other activate only the cellular components of the immune system, like phagocytic function, without affecting humoral or cell-mediated immunity. Indian medicinal plants are a rich source of substances, which are claimed to induce part immunity, the non-specific immunomodulation of especially granulocytes, macrophages, natural killer cells and competent function. Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health a concept of strengthening host defense system.
thereby attaining resistance against different diseases\textsuperscript{19}. In Ayurveda, \textit{T. cordifolia} and its preparations have been routinely used to boost the immune system and the resistance against infections. Moreover, recent investigations were also attributed the immunodulatory properties to the \textit{T. cordifolia}. Pretreatment with both ethanolic and aqueous dried stem extract of \textit{T. cordifolia} to mice reported to have immunomodulatory activity, suppressed by cyclophosphamide\textsuperscript{20}.

It was observed that the description of Guduchi in Ayurvedic literature matches more closely with \textit{T. sinensis} rather than with \textit{T. cordifolia} (Table 1). Unfortunately, the correct identification of Guduchi as described in Ayurveda is elusive. This is due to the similar characters viz. habit, lenticels present on stem, appears wheel like shape when cut transversely, bitter taste of stem or \textit{Satwa}, shape of leaf, flower and fruit color. However, \textit{T. cordifolia} is easily available and hence used in major proportion. Also, in ancient Ayurvedic treatise, it was reported that \textit{T. cordifolia} growing on \textit{Azadirachta indica} A. Juss (\textit{Neem}) commonly called as \textit{Neem-guduchi} is more potent (immunostimulating) than \textit{T. cordifolia} and \textit{T. sinensis}\textsuperscript{21,22}.

Considering these facts, the present study has been designed to evaluate comparative immunomodulatory potential of \textit{Tinospora cordifolia}, \textit{Tinospora sinensis} and \textit{Neem}-guduchi. It is of utmost interest to identify most potent guduchi through preparing a formulation called “Guduchi \textit{Satwa}” as described in Ayurveda as well as to enhance the efficacy of Ayurvedic formulation for particular ailments.

**Materials and Methods**

**Collection of plant material**—Stems of selected species were collected during November 2010 from Pune, India. The plants were identified and voucher specimen has been deposited at the herbarium of Medicinal Plants Conservation Center, Pune; \textit{Tinospora cordifolia} (Willd.) Miers ex Hook. F. & Thoms (MPCC 3464), \textit{Tinospora sinensis} (Lour.) Merr. (MPCC 3525) and \textit{Neem}-guduchi (\textit{T. cordifolia} (Willd.) Mier s ex Hook. F. & Thoms) (MPCC 3526).

**Preparation of Guduchi \textit{Satwa}**—Fresh stems of selected three variants of \textit{Tinospora} sp. were used for the preparation of \textit{Guduchi \textit{Satwa}}. The preparation was defined in Ayurvedic literature as sediment extract, which is predominantly starchy in nature. In brief, freshly collected stem parts were washed with water and cut into small pieces. They were hand-macerated in water and left overnight to sediment. Next morning, the water was decanted, solid part that remained at bottom was then air dried when ripe and also planted. After drying, the dry material was ground to a fine powder, which was collected as Guduchi \textit{Satwa}\textsuperscript{23}. This \textit{Satwa} was resuspended in water at the time of oral administration.

**Experimental animals**—The study was carried out on male Wistar rats (150–250 g). Animals were maintained under standard husbandry conditions 25±2 °C, 12: 12-h L.D cycle and fed with standard pellet diet (Amrut, Sangali, M.S., India) and tap water \textit{ad libitum}. All animal experiments were handled according to the international guidelines for the care and use of laboratory animals of National Research Council (1996). This study was carried out in accordance with CPCSEA guidelines. The study was

<table>
<thead>
<tr>
<th>No.</th>
<th>Character</th>
<th>\textit{T. cordifolia}</th>
<th>\textit{T. sinensis}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Habit</td>
<td>Extensive climber</td>
<td>Straggling shrubs</td>
</tr>
<tr>
<td>2.</td>
<td>Stem</td>
<td>With lenticels</td>
<td>With lenticels</td>
</tr>
<tr>
<td>3.</td>
<td>Bark</td>
<td>Green &amp; corky</td>
<td>bark dirty green, wary</td>
</tr>
<tr>
<td>4.</td>
<td>T.S. of stem</td>
<td>Wheel like shape</td>
<td>Wheel like shape</td>
</tr>
<tr>
<td>5.</td>
<td>Taste</td>
<td>Bitter</td>
<td>Bitter</td>
</tr>
<tr>
<td>6.</td>
<td>Leaves size</td>
<td>5.0 – 8.5 cm</td>
<td>8–12 cm</td>
</tr>
<tr>
<td>7.</td>
<td>Leaf proportion</td>
<td>Broad as long</td>
<td>Long as broad or broader than long</td>
</tr>
<tr>
<td>8.</td>
<td>Leaves shape</td>
<td>Ovate reniform</td>
<td>Ovate of cordate</td>
</tr>
<tr>
<td>9.</td>
<td>Leaf hairs</td>
<td>Non hairy</td>
<td>Dense hairy</td>
</tr>
<tr>
<td>10.</td>
<td>Leaf width</td>
<td>Thin papery</td>
<td>Thick leathery</td>
</tr>
<tr>
<td>11.</td>
<td>Leaf color</td>
<td>Dark green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>12.</td>
<td>Leaves number</td>
<td>More (up to 10 per feet)</td>
<td>Less (up to 4 per feet)</td>
</tr>
<tr>
<td>13.</td>
<td>Petiole</td>
<td>3-4 cm long</td>
<td>8-11 cm long</td>
</tr>
<tr>
<td>14.</td>
<td>Branches</td>
<td>Wiry long</td>
<td>Thickly short</td>
</tr>
<tr>
<td>15.</td>
<td>Flowers</td>
<td>Greenish – yellow</td>
<td>Greenish – yellow</td>
</tr>
<tr>
<td>16.</td>
<td>Flower size</td>
<td>5-8 mm across</td>
<td>5-7 mm across</td>
</tr>
<tr>
<td>17.</td>
<td>Flowers male</td>
<td>Fascicled</td>
<td>In racemes from bare branches</td>
</tr>
<tr>
<td>18.</td>
<td>Flowers female</td>
<td>Solitary or in raceme</td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>Drupe size</td>
<td>5-6 mm across</td>
<td>09-1.2 cm across</td>
</tr>
<tr>
<td>20.</td>
<td>Drupe color</td>
<td>Drupe orange-red when ripe</td>
<td>Drupe orange-red when ripe</td>
</tr>
<tr>
<td>21.</td>
<td>Drupe shape</td>
<td>Globose</td>
<td>Ellipsoidal</td>
</tr>
<tr>
<td>22.</td>
<td>Flower &amp; fruiting time</td>
<td>January - August</td>
<td>January - May</td>
</tr>
<tr>
<td>23.</td>
<td>Distribution</td>
<td>Common in deciduous forests and also planted</td>
<td>Scattered, infrequent in deciduous forests.</td>
</tr>
<tr>
<td>24.</td>
<td>First botanically identified in India</td>
<td>1806</td>
<td>1934</td>
</tr>
</tbody>
</table>
Neutrophil adhesion test—The test was carried out according to the method of Fulzele et al\textsuperscript{24}. Rats were divided into 7 groups of 6 each. Group I (control) animals received normal saline for 7th days. Groups II and III received \textit{T. cordifolia} at 150 and 300 mg/kg, po dose respectively for 7th days. Groups IV and V received \textit{T. sinensis} at 150 and 300 mg/kg, po dose respectively for 7th days. On the 7th day of drug treatment, blood samples were collected from retro-orbital plexus (under light anesthesia) of all rats into heparinized vials and analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC). The product of TLC and DLC of blood sample was divided by the neutrophil counts due to adhesion of neutrophil to the fibers. Neutrophils adhesion test (Table 2) is indicative of the marginalization of phagocytic cells in the blood vessels, an indication of immuno-stimulation. The percent neutrophil adhesion in control group animals was 15.26±1.25, in \textit{Neem-guduchi Satwa} treated group animals at dose 300 mg/kg, po was 23.52±0. Neem-Guduchi Satwa showed a significant increase in neutrophil adhesion (\textit{P}< 0.01) at a dose of 300 mg/kg, po day in rats. There was no significant increase in neutrophil adhesion at a dose of 150 mg/kg, po day in \textit{Tinospora cordifolia satwa} and Neem-guduchi Satwa.

Effect on haemagglutinating antibody titre—For haemagglutinating antibody (HA) titre, the animals were treated with \textit{guduchi satwa} for 7 days and blood samples were analyzed from each rat on day 7th for HA titre and \textit{Neem-guduchi} (300 mg/kg, po) treated.
group showed significant increases (5.66±0.76) in HA titre when compared with control group (2.83±0.16) suggesting possible immunostimulation action of the Neem-guduchi at dose 300 mg/kg, po (Fig. 1).

**Effect on delayed type hypersensitivity (DTH) response**—The DTH response using SRBC as an antigen, on 7th day after 24 h of challenge in control group animals was (0.0483±0.01) while in Neem-guduchi treated group animals at dose 300 mg/kg, po was 0.2150±0.139. Statistically most significant (*P*<0.01) compared to control.

Thus, it can be said that Neem-guduchi Satwa induced marked enhancement in DTH response to SRBC in the animals (Fig. 2).

**Discussion**

Many exogenous and endogenous factors influence the function and efficiency of the immune system. In the development of immunotherapy use of immunomodulators has a vital place. These immunomodulators of either plant or animal origin enhance the immune responsiveness of body against pathogens by activating the non-specific immune system. Thus, use of medicinal plant products as a possible therapeutic measure for immunomodulation has become a subject of active scientific investigations.

In the present study, Neem-guduchi at the dose of 300 mg/kg, po, stimulate cell mediated immunity as shown by an increase in neutrophil adhesion to nylon fibres and decrease in DTH reactions. It also stimulated humoral immunity as indicated by an increase in serum immunoglobulin levels.

In the present study, Neem-guduchi Satwa significantly evoked neutrophil adhesion to nylon fibres marked as increase in adhesion percentage (Table 2). This directly correlates with circulation of neutrophils in the vasculature in a passive state and become more adhesive upon stimulation at sites of inflammation, while it was margnated to the vessel wall, subsequently by transmigration and phagocytosis. This adhesion could be attributed to upregulation of β2 integrins, on the membrane of the

**Table 2**—Effects of aqueous stem extract of *T. cordifolia*, *T. sinensis* and Neem-guduchi neutrophil adhesion in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TLC (cell/mm³) (A)</th>
<th>Neutrophil (%) (B)</th>
<th>Neutrophil Index (A × B)</th>
<th>Neutrophil adhesion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UB FTB</td>
<td>UB FTB</td>
<td>UB FTB</td>
<td>UB FTB</td>
</tr>
<tr>
<td>Control</td>
<td>4500± 400</td>
<td>4150± 250</td>
<td>55.5± 3.5</td>
<td>51± 1</td>
</tr>
<tr>
<td><em>T. cordifolia</em> (150 mg/kg, po)</td>
<td>5250± 1350</td>
<td>4700± 900</td>
<td>42.5± 3.5</td>
<td>39.5± 3.5</td>
</tr>
<tr>
<td><em>T. cordifolia</em> (300 mg/kg, po)</td>
<td>5350± 1050</td>
<td>5150± 1150</td>
<td>43± 1</td>
<td>40.5± 0.5</td>
</tr>
<tr>
<td><em>T. sinensis</em> (150 mg/kg, po)</td>
<td>5000± 378.5</td>
<td>4733.33± 328.29</td>
<td>49± 7.3</td>
<td>45.66± 1.76</td>
</tr>
<tr>
<td><em>T. sinensis</em> (300 mg/kg, po)</td>
<td>3600± 0</td>
<td>3500± 0</td>
<td>38± 0</td>
<td>36± 0</td>
</tr>
<tr>
<td>Neem-guduchi (150 mg/kg, po)</td>
<td>5900± 0</td>
<td>5200± 0</td>
<td>53± 0</td>
<td>50± 0</td>
</tr>
<tr>
<td>Neem-guduchi (300 mg/kg, po)</td>
<td>6300± 0</td>
<td>4900± 0</td>
<td>63± 0</td>
<td>49± 0</td>
</tr>
</tbody>
</table>

*P*<0.01; TLC = Total leukocytes count, UB = Untreated blood, FTB = Fiber treated blood
neutrophils. The neutrophilic phagocytic system plays an important role in host immune mechanism. Thus, it can be suggested that immunomodulation could be attained through increased neutrophil adhesion stimulated by Neem-guduchi Satwa when compared to the control.

The haemagglutination antibody titre was used to assess humoral immune response. This test was performed to estimate serum immunoglobulin levels to evaluate increase in serum immunoglobulin production after the administration of the drug. The augmentation of HI response to SRBCs by Satwa of all three Tinospora species evidenced by increase in the antibody titre in the blood of rats. Major immunoglobulins namely IgG and IgM are central to humoral immune responses, which are involved in the complement activation, opsonization, neutralization of toxins, etc. The anti-SRBC antibody titre was raised in Neem-guduchi satwa treated groups in dosage of 150, 300 mg/kg with normal immune status, but was found statistically significant only at the dose of 300 mg/kg, when compared to control group.

Increased DTH response of Neem–guduchi Satwa is directly associated with cell mediated immunity (CMI). CMI responses are critical to defense against infectious organisms, infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions. DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines resulting in increased vascular permeability. Macrophage accumulation induces vasodilatation, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. When these activated cells encounter certain antigens, viz. SRBCs, they secrete cytokines that induce inflammatory reaction called delayed type hypersensitivity reaction. The delay in the onset of the response reflects the time required for the cytokines to induce the recruitment and activation of macrophages. Therefore, increase in DTH reaction in mice in response to T cell dependent antigen revealed the stimulatory effect of Neem-guduchi Satwa on T cells.

It can be thus postulated that immunostimulatory effect produced by Neem-guduchi satwa may be due to cell mediated and humoral antibody mediated activation of T and B cells as initially reported by Bhalerao et al. Moreover, it accordingly supports the claim of ancient Ayurvedic literature. This action could be attributed to exchange of bioactive constituents from A. indica to T. cordifolia but further studies are warranted to elucidate the exact mechanisms responsible for the observed immunomodulation.

Earlier researcher demonstrated that ethanol and methanol stem extracts of T. cordifolia (100, 200 mg/kg, po respectively) appears to enhance total count of leucocytes in mice. Additionally, it also stimulates dose dependant humoral immune response significantly indicating stimulatory effect on hematopoietic system. In summary, the observed resultant on immunomodulatory potential is in the order T. sinensis < T. cordifolia < Neem-guduchi.

It can therefore be concluded that Satwa of Neem-guduchi has shown the immunostimulatory activity compared with T. cordifolia and T. sinensis by potentiating humoral as well as cellular immunity, also through increasing adhesion in neutrophils. Therefore it may be used as a complimentary therapeutic agent. Similarly, it has been recommended in different immunomodulatory formulations in which Guduchi is advised to be used. Apart from all of these properties it needs further comparative characterization of chemical constituents of each plant to know potent immunomodulating components along with their proportionate combination and changes in mechanism of action when grows on Neem tree. Even clinical studies with Neem-guduchi as immunostimulant is further justified and can assume that it will result in positive outcome.

References
2. Weil (Chopra RN, Nayar SL & Chopra IC, Place, Dehra Dun, India) 1975.


