EFFECT OF EDAPHIC FACTORS ON MAJOR SECONDARY METABOLITES OF *TINOSPORA CORDIFOLIA* AND NEEM GUDUCHI WITH RESPECT TO THEIR IMMUNOMODULATORY EFFECT

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**Keywords:**
*Tinospora cordifolia*, Neem guduchi, Satwa, Immunomodulation, Edaphic factors

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**ABSTRACT:** *Tinospora cordifolia* is a requisite medicinal plant, has been used for the treatment of various diseases and has been recommended for improving the immune system mentioned in Ayurveda and other systems of medicine as a monoherbal or polyherbal preparation. Nonetheless, Ayurvedic and other ancient literature reported that *Tinospora cordifolia* growing on neem plant gives better immunomodulatory activity. The traditional and scientific data comprising immunomodulatory potential, pharmacognostic description, therapeutic uses, phytochemical constitution, of *Tinospora cordifolia* and neem guduchi using the aqueous extract in the form of satwa has well established. The present study evaluates the comparative immunomodulatory activity using satwa of *Tinospora cordifolia* and neem guduchi using *in-vitro* models, for understanding the influence of edaphic factors on biologically active compounds. Evaluation of the immuno-modulatory potential of the guduchi satwa and neem guduchi satwa at the dose of 150 and 300 mg/kg body weight was done in rats; assessment of immunomodulatory activity was carried out by assessment of neutrophil adhesion whereas phytochemical profile was established by HPTLC method. Simultaneously, the correlation between soil factors and medicinally active compound responsible for immunomodulation also studied. In the present study, we have compiled and investigated the active compounds which are immunomodulator. Results of present studies suggested that neem guduchi satwa was found to be potent immuno-stimulant in a dose-dependent manner when compared with other group and there is no clear correlation between soil factors and medicinally active compound were observed.

**INTRODUCTION:** During the past 15 years, pharmaceutical industry research into natural products has declined because lots of challenges face in drug discovery from natural sources, most of the current difficulties are categories:

The prevailing paradigm for drug discovery in large pharmaceutical industries, emphasis on high-throughput screening of synthetic libraries. Secondary metabolites from medicinal plants are unique sources for pharmaceuticals, food additives, flavors, and industrially important bio chemical’s. Due to the rapid increase in the uses of medicinal plants and herbal remedies over the world, in recent years, demand for these plants has significantly increased. A majority of new drugs have been generated from natural products. In advanced countries, alternative medicine is now recognized...
as an invaluable resource even by the most intransigent clinicians. The immune system plays an important role in biological adaptation, contributing to the maintenance of homeostasis. Plants have been widely investigated for their possible immunomodulatory properties.

Hence, the plant products have long been used as immunomodulators by the traditional healers, and one of the most promising recent alternatives to classical antibiotic treatment is the use of immunomodulators for enhancing host defense responses. A number of natural products and synthetic immunopotentiators termed as Biological Response Modifiers (BRMs) are becoming increasingly popular for testing their potential for augmenting immune responses. Among the natural BRMs many herbs and medicinal plants have long been known for their immunologic potential, however, only recently scientists have recognized them for their possible BRM actions. The satwa isolated from botanical sources, Tinospora spp. has attracted a great deal of attention in the biomedical ground because of its broad spectrum of therapeutic properties and relatively low toxicity.

In Ayurveda, Tinospora cordifolia and its preparations have been routinely used to boost the immune system and the resistance against infections. Tinospora cordifolia (Willd.) Hook. f. and Thoms. (Guduchi) is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae is one of the major constituent of several Ayurvedic preparations used preferably for general debility, dyspepsia, fever, urinary diseases etc. But interestingly it is mentioned in ancient literature such as Rigveda, Atharvaveda, it was reported that that Guduchi that grows on neem (Azadirachta indica A. Juss.) tree has a better potential and preferentially used in treatment of certain diseases, presumably due to close vicinity to Neem. The quantitative estimation of the concentration of various trace elements is important for determining the effectiveness of the medicinal plants in treating various diseases and in understanding their pharmacological action.

Moreover, this guduchi possesses a wide range of active compounds like berberine, protoberberine, palmatine, magnoflorine, tinosporin, etc. Berberine is an isoquinoline alkaloid and is mainly found in the stem and minor quantities in roots also. However, the distribution of metabolites in the root parts was mainly affected by variation in edaphic factors.

Thus, research is necessary to study the probable reason for the enhancement of the activity when the plant comes in association with neem tree and the influence of soil nutrients. Therefore, in the present study, the comparison will be carried out between Tinospora cordifolia (Willd.) Hook. f. and Thoms and neem guduchi (T. cordifolia growing on neem tree) satwa in terms of their soil nutrients, active compounds, and immunomodulatory effect.

MATERIALS AND METHODS:

**Plant Material:** The stem of Tinospora cordifolia and Tinospora cordifolia association with neem (A. indica) was freshly collected from two places Rawet, Tal- Mulshi, and vicinity of Vellhe Tal-Vellhe Maharashtra, India during November-December 2016. The plant stems were authenticated by Dr. Suresh D. Jagtap, Taxonomist and herbarium was deposited in Herbaria of Medicinal Plants Conservation Centre, Pune (MPCC3464). Soil samples were collected from near about one Ft. depth around roots of the plants (more than one Kg weight).

**Preparation of Plant Extract:** The collected stems of Tinospora cordifolia and neem guduchi were washed and stem was chopped in one inch pieces. After that, little damage or crush the material and finally kept in normal water for soaking for overnight. Whitish, little sticky, starchy material was settled down in bottom. That is called as satwa was collected by removing the debris of plant material. Now watery content was removed by evaporation in hot air oven at 50-70 °C. The satwa was preserved for biochemical analysis and for animal experiments, the satwa was reconstituted in water to get the desired dose.

**Comparative Evaluation of Immunomodulatory Potential of T. cordifolia and Neem Guduchi:**

**Animals:** This study was carried out on male Wister rat. Seven to eight weeks old Male Wister rats weighing about 150-200 gm. Animals divided into 7 groups with 6 rats each were used for the study. Animals were procured from National Institute of Biosciences, Pune.
The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25 ± 30 °C and 35-60% humidity). Standard palletized feed and tap water were provided ad libitum. The study was approved by the Institutional Animal Ethical Committee of Medical College, Bharati Vidyapeeth Deemed University Pune, India registered under CPCSEA India. (No: 14/139/15 CPCSEA).

Dosage and Concentrations: Treatment with the satwa of *Tinospora cordifolia* and neem guduchi was given orally. For the preparation of drug solutions, sterile saline was used. As a standard drug, Prednisolone at a dose of 5 mg/ kg body weight was given by orally. Group, I received only water and served as control. Group II received standard drug and antigen, served as positive control, Group III received as the only antigen, served as negative control, Group IV, V served as 150 and 300 mg/ kg body weight of satwa of *Tinospora cordifolia* and Group VI, VII served as 150 and 300 mg/ kg body weight satwa of neem guduchi.

Antigen: Fresh sheep blood was collected from a local slaughterhouse in a sterilized container in the presence of Alsever’s solution in 1:1 proportion kept in the refrigerator. Sheep red blood cells (SRBC) for immunization were obtained by centrifugation of sheep blood at 2000 rpm for 10 min, and the cells were washed 3 times in saline and suspended it in the desired concentration of administration 0.4 ml of 5×10^9 SRBCs/ml by intraperitoneal for immunization and challenge 10.

Assessment of Neutrophil Index and Neutrophil Adhesion in Rats: Neutrophil adhesion test was performed according to the method described by Ismail and Asad, 2009 11. Rats divided into 7 groups were treated orally either with vehicle (water in present study or drug (Satwa, prednisolone) for seven days. On the 7th day of drug treatment, blood samples were collected from retro-orbital plexus into heparinized vials analysis for TLC (Total leukocyte count) and DLC (Differential leukocyte count) by fixing blood smears and staining with field stain 1 and Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fiber for 15 min at 38 °C. The incubated blood samples were analyzed for TLC and DLC. The product of TLC and % neutrophil gives a neutrophil index of blood samples. Percent of neutrophil adhesion was calculated as shown below:

\[ \text{Neutrophil adhesion (\%)} = \frac{\text{NI}_U - \text{NI}_T}{\text{NI}_U} \times 100 \]

Where, NI_U- Neutrophil index of the untreated blood sample, NI_T- Neutrophil index of the treated blood sample.

Evaluation of Delayed Type Hypersensitivity (DTH) Response: A method described by Bhalerao et al., 2012 12 to measure delayed-type hypersensitivity response in the rat as a function of treatment. On the day ‘0’, animals in all the groups were immunized by intraperitoneal administration of 0.5 × 10^9 cells/ml of SRBCs suspension per rat. On day 7, all the three groups of rats were challenged with 0.1 ml of 0.5 × 10^9 cells/ml SRBCs in right hind paw, while contralateral paw was injected with an equal volume of normal saline and served as control. The thickness of both paws in case of all the animals was measured at 0, 24 h plethysmometrically using digital Plethysmometer (Ugo-Basile 7140, Italy). The difference in the volume of right paws at 0 h and 24 h was used as a measure of DTH response.

Determination of Humoral Immune Response: Indirect hemagglutination test was used to measure humoral immune response 13. Each pretreated rat, including the control group, was immunized with 5×10^9 cells by an intraperitoneal route at day zero. The treatment of guduchi satwa and neem guduchi satwa at 150 and 300 mg/kg body weight was continued for next seven days, and blood samples were collected individually from retro-orbital plexus on the seventh day for the determination of haemagglutinating antibody (HA) titer. The titer value was determined by titrating serum dilution with SRBC (1.25 × 10^9 cells) in the microtitre plate. The reaction mixture was incubated at room temperature for overnight and usually examined for agglutination. The highest serum dilution showing haemagglutination was noted and expressed as HA titer.

Statistical Analyses: The statistical analysis was assessed using one-way ANOVA followed by Dunnett comparison test using graph pad prism 5.00. All values are expressed as Mean ± S.E.M.
**Soil Sample:** Soil samples were taken from agro-ecological zones like the area of near the plant root, from 30-45 cm depths. Soil samples were collected and air dried and sieved to pass through before analysis.

**Soil Test:** Analysis of soil samples was done by using various parameters like pH of the soil, micro and macro elements in soil, sand and silt concentration, etc. Soil samples were analyzed in Technext Laboratory Pune, India.

**HPTLC:** Both satwa is separated and estimated quantitatively on pre-coated silica gel 60 G254 plates with toluene: acetone: water (2.5: 7.5: 0.5) using the densitometric scanner at 220 nm. The chromatogram was developed in glass twin-trough chambers (10 cm × 10 cm, with metal lids; Camag, Switzerland) previously saturated with mobile phase vapour for 20 min. The development distance was 8 cm. After the development of High-Performance Thin - Layer Chromatography (HPTLC), the plate was removed from the chamber. To develop HPTLC fingerprint chromatogram, scanning was performed on Camag TLC scanner III under UV 220 nm.

**RESULTS:**

**Effect on Neutrophil Adhesion Test:** Incubation of neutrophils with nylon fibers produced a decrease in the neutrophil counts due to adhesion of neutrophil to the fibers. A neutrophils adhesion test is indicative of the marginalization of phagocytic cells in the blood vessels, an indication of immunostimulation. TCN (300 mg/kg) and TC (150 mg/kg) treated animal having neutrophil adhesion value 61.6 ± 9.33 and 52.45 ± 3.9 respectively Fig. 1, this show increase in neutrophil adhesion test compared to other treated groups as well as with prednisolone treated animals. Thus among them neem guduchi satwa treated group animals at dose 300 mg/kg, p.o. showed maximum neutrophil adhesion. Simultaneously, hemoglobin and total RBC count was also estimated.

**Determination of Haemoglobin And Red Blood Cell Count:** Neem guduchi (300 mg/kg/p.o.) treated group showed increase (12.3 ± 0.543 gm %) in Hb count compared to prednisolone treated group (11.9 ± 0.602 gm %), followed by TC (300 mg/kg) and TC (150 mg/kg) treated group i.e. 11.4 ± 0.736 gm % and 11.1 ± 0.2 gm % respectively. TCN (300 mg/kg) treated animals showed the same effect when compared to healthy control Table 1. Similarly, RBC count of neem guduchi (300 mg/kg/p.o.) treated group showed an increase (8.2 ± 0.25 106/µl) compared to other treated group.

![Neutrophil adhesion test](image)

**TABLE 1: EFFECT OF T. CORDIFOLIA AND NEEM-GUDUCI ON HEMOGLOBIN AND RED BLOOD CELL COUNT**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Hb count (gm %)</th>
<th>RBC Count (10×10^6/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>HC</td>
<td>12.1 ±0.368</td>
<td>8.2 ± 0.668</td>
</tr>
<tr>
<td>II</td>
<td>NC</td>
<td>10.2 ±0.776</td>
<td>7.2 ± 0.441</td>
</tr>
<tr>
<td>III</td>
<td>Prednisolone</td>
<td>11.9 ± 0.602</td>
<td>8.1 ±0.694</td>
</tr>
<tr>
<td>IV</td>
<td>TC 150 mg/kg</td>
<td>11.1 ±0.2</td>
<td>7.7 ±0.206</td>
</tr>
<tr>
<td>V</td>
<td>TC 300 mg/kg</td>
<td>11.4 ± 0.736</td>
<td>7.8 ± 0.330</td>
</tr>
<tr>
<td>VI</td>
<td>TCN 150 mg/kg</td>
<td>10.8 ± 0.805</td>
<td>7.5 ± 0.818</td>
</tr>
<tr>
<td>VII</td>
<td>TCN 300 mg/kg</td>
<td>12.3 ±0.543</td>
<td>8.2 ± 0.25</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=7, compared with the negative control. *P< 0.01 and **P<0.001 HC=Healthy control, NC= Negative control, TCL=Tinospora cordifolia, TCN=Tinospora cordifolia neem guduchi.

**Effect on Humoral Immune Response:** The haemagglutination antibody titer was used to assess humoral immune response. Neem guduchi (300 mg/kg/p.o.) treated group showed maximum increases (5.2 ± 0.74) in humoral immune response when compared with prednisolone treated group (4.2 ± 0.4). Also, Neem guduchi (300 mg/kg/p.o.) treated group showed a significant increase in
humoral immune response compared to TCL (150 mg/kg), TC (300 mg/kg), and TCN (150 mg/kg) treated groups Fig. 2.

**Effect on Delayed Type Hypersensitivity Response (DTH Response):** SRBCs induced delayed-type hypersensitivity was used to assess the effect of satwa on cell-mediated immunity. In Prednisolone treated group DTH response was 0.26 ± 0.127, which is less as compared to neem guduchi treated group animals at dose 300 mg/kg p.o. was 0.29 ± 0.08. TC (300 mg/kg) and TCN (150 mg/kg) treated group animal show same DTH response Fig. 3. So, DTH response in neem guduchi treated group animals at dose 300 mg/kg p.o. was highest and statistically significant.

By summarizing above, all the results in Table 1, the evaluation of the comparative immunomodulatory effect of *Tinospora cordifolia* and neem guduchi satwa were studied by assessing different parameters. In all the parameters animals which are treated with neem guduchi satwa at a dose of 300 mg/kg showed a significant increase when compared to all other treated group Table 2. Thus neem guduchi satwa shows maximum immunomodulatory activity.

**TABLE 2: COMPARATIVE IMMUNOMODULATORY EFFECT OF *T. CORDIFOLIA* SATWA AND NEEM GUDUCHI SATWA ON NEUTROPHIL ADHESION, HEMOGLOBIN COUNT, RED BLOOD CELL, HAEMAGGLUTINATION IMMUNE RESPONSE, DELAYED-TYPE HYPERSENSITIVITY**

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Groups</th>
<th>Neutrophil adhesion test</th>
<th>Hemoglobin count</th>
<th>Red blood cell</th>
<th>Haemagglutination titre</th>
<th>Delayed-type hypersensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Healthy control</td>
<td>55.9 ±4.65</td>
<td>12.1 ± 0.36</td>
<td>8.2 ± 0.66</td>
<td>1.4 ± 0.48</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>Negative control</td>
<td>43 ± 12.32</td>
<td>10.2 ± 0.77</td>
<td>7.2 ± 0.44</td>
<td>3.6 ± 0.48</td>
<td>0.12 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>Prednisolone</td>
<td>47.9 ± 13.97</td>
<td>11.9 ± 0.60</td>
<td>8.1 ± 0.69</td>
<td>4.2 ± 0.4</td>
<td>0.26 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td><em>T. cordifolia</em> (150 mg/kg)</td>
<td>52.45 ± 3.93</td>
<td>11.1 ± 0.2</td>
<td>7.7 ± 0.206</td>
<td>3.6 ± 0.99</td>
<td>0.18 ± 0.09</td>
</tr>
<tr>
<td>5</td>
<td><em>T. cordifolia</em> (300 mg/kg)</td>
<td>32.29 ± 2.81</td>
<td>11.4 ± 0.73</td>
<td>7.8 ± 0.33</td>
<td>3.2 ± 0.51</td>
<td>0.27 ± 0.10</td>
</tr>
<tr>
<td>6</td>
<td>Neem guduchi (150 mg/kg)</td>
<td>41.75 ± 5.59</td>
<td>10.8 ± 0.80</td>
<td>7.5 ± 0.81</td>
<td>3.8 ± 0.74</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>Neem guduchi (300 mg/kg)</td>
<td>61.6 ± 9.33</td>
<td>12.3 ± 0.54</td>
<td>8.2 ± 0.25</td>
<td>5.2 ± 0.74</td>
<td>0.29 ± 0.08</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=7.

**Soil Test:** Following the table, Table 3 shows the parameter studied for the soil collected from different locations. By summarizing above, the value of organic carbon in *Tinospora cordifolia* soil is 4.60 more than neem guduchi plant soil 2.22. Total iron content in *Tinospora cordifolia* plant soil contains 21.95 is more than neem guduchi 9.40. The copper content in *Tinospora cordifolia* 12.73 ppm than neem guduchi 5.10 Table 3.

Other parameters showed nearly similar content values finally we concluded that presence of availability of organic carbon, iron content, and copper content is less in neem guduchi soil than *Tinospora cordifolia* soil. Reduction availability of this content in *Neem* guduchi soil is responsible for the presence of unknown but beneficially compound acts as immunomodulator or immunostimulator.
### TABLE 3: SOIL PARAMETER STUDY REPORT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Specification</th>
<th>Remark</th>
<th>Neem Guduchi</th>
<th>T. cordifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.51-5.50</td>
<td>Acidic</td>
<td>6.61</td>
<td>6.41</td>
</tr>
<tr>
<td></td>
<td>6.51-7.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.51-8.50</td>
<td>Normal pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conductivity ds/m</td>
<td>Less than 1.00</td>
<td>Normal</td>
<td>0.36</td>
<td>0.19</td>
</tr>
<tr>
<td>Free lime %</td>
<td>Less than 0.50</td>
<td>Very Less</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>1.01-2.00</td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.01-5.00</td>
<td>More than required</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic carbon%</td>
<td>0.81-1.00</td>
<td>High</td>
<td>2.22</td>
<td>4.60</td>
</tr>
<tr>
<td></td>
<td>More than 1.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Available phosphorous Kg/hector</td>
<td>Less than 7.00</td>
<td>Very less</td>
<td>17.92</td>
<td>13.44</td>
</tr>
<tr>
<td></td>
<td>07.01-14.00</td>
<td>Less</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.01-21.00</td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Available Nitrogen Kg/hector</td>
<td>281-420</td>
<td>Normal</td>
<td>470.4</td>
<td>672.0</td>
</tr>
<tr>
<td></td>
<td>421-560</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>561-700</td>
<td>More than required</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Available potassium Kg/hector</td>
<td>Less than 100</td>
<td>Very Less</td>
<td>672.0</td>
<td>560.0</td>
</tr>
<tr>
<td></td>
<td>More than 301</td>
<td>Very high</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium ppm</td>
<td>0501-1000</td>
<td>Normal</td>
<td>4725.0</td>
<td>3750.0</td>
</tr>
<tr>
<td></td>
<td>1001-5000</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium ppm</td>
<td>Less than 250</td>
<td>Less</td>
<td>1325.0</td>
<td>1175.0</td>
</tr>
<tr>
<td></td>
<td>More than 1001</td>
<td>Very High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphar ppm</td>
<td>11-50</td>
<td>Normal</td>
<td>329.0</td>
<td>259.0</td>
</tr>
<tr>
<td></td>
<td>More than 101</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total iron ppm</td>
<td>2.10-4.50</td>
<td>Normal</td>
<td>9.40</td>
<td>21.95</td>
</tr>
<tr>
<td></td>
<td>More than 4.51</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese ppm</td>
<td>1.10-2.00</td>
<td>Normal</td>
<td>17.65</td>
<td>24.28</td>
</tr>
<tr>
<td></td>
<td>More than 2.01</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zink ppm</td>
<td>0.51-1.00</td>
<td>Normal</td>
<td>2.15</td>
<td>3.38</td>
</tr>
<tr>
<td></td>
<td>More than 1.01</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper ppm</td>
<td>0.21-1.00</td>
<td>Normal</td>
<td>5.10</td>
<td>12.73</td>
</tr>
<tr>
<td></td>
<td>More than 1.01</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium ppm</td>
<td>Less than 1000</td>
<td>Safe</td>
<td>250</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>More than 1001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cation exchange Ratio Meq/100g</td>
<td>---</td>
<td>--</td>
<td>36.53</td>
<td>42.88</td>
</tr>
<tr>
<td>Sand %</td>
<td>---</td>
<td>--</td>
<td>24.0</td>
<td>22.20</td>
</tr>
<tr>
<td>Silt %</td>
<td>--</td>
<td>--</td>
<td>48.0</td>
<td>53.00</td>
</tr>
</tbody>
</table>

**HPTLC Characterization of Guduchi and Neem Guduchi:** Following Fig. 4 shows the HPTLC profile of both guduchi and neem guduchi using it satwa.

![A) HPTLC FINGERPRINT OF GUDUCHI SATWA (10 mg/ml, w/v)](image1)

![B) HPTLC FINGERPRINT OF NEEM GUDUCHI SATWA (10 mg/ml, w/v)](image2)

**FIG. 4: HPTLC PROFILE OF GUDUCHI AND NEEM GUDUCHI AT 220 nm**
TABLE 4: HPTLC PROFILE OF GUDUCHI AND NEEM GUDUCHI AT 220 nm WITH Rf VALUE AND AREA UNDER THE PEAK

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Guduchi Rf value</th>
<th>Area under the peak</th>
<th>Peak number</th>
<th>Neem Guduchi Rf value</th>
<th>Area under the peak</th>
</tr>
</thead>
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<tr>
<td>1</td>
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<td>1131.7</td>
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<td>939.3</td>
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<tr>
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<td>0.33</td>
<td>847.2</td>
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<td>0.03</td>
<td>2255.1</td>
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<tr>
<td>3</td>
<td>0.41</td>
<td>11330.0</td>
<td>3</td>
<td>0.29</td>
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<tr>
<td>4</td>
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<td>197.1</td>
<td>4</td>
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<tr>
<td>5</td>
<td>0.70</td>
<td>125.9</td>
<td>5</td>
<td>0.41</td>
<td>10810.7</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>0.80</td>
<td>1306.7</td>
</tr>
<tr>
<td>7</td>
<td>0.86</td>
<td>244.6</td>
<td>7</td>
<td>0.85</td>
<td>152.9</td>
</tr>
<tr>
<td>8</td>
<td>0.97</td>
<td>384.9</td>
<td>8</td>
<td>0.97</td>
<td>524.6</td>
</tr>
</tbody>
</table>

Comparative Spectra:

HPTLC Profile of Guduchi and Neem Guduchi Satwa: The results of HPTLC profile of guduchi and neem guduchi aqueous satwa shows HPTLC fingerprint at 220 nm with 7 spots at Rf 0.03, 0.33, 0.41, 0.55, 0.70, 0.86, and 0.97 and 8 spot at Rf 0.02, 0.03, 0.29, 0.32, 0.41, 0.80, 0.85, 0.97 respectively.

Fig. 5. However, in neem guduchi, the sixth spot shows an additional peak at Rf 0.80 having area 1306.7 Table 4. This may be due to the additional component present in the neem or due to the association between Tinospora cordifolia and neem.

DISCUSSION: T. cordifolia reported to have beneficial effects on the immune system by using their aqueous and alcoholic extracts. In the present study, the comparative study of immunomodulatory effect by preparing of Tinospora cordifolia (Willd.) Hook. F. and Thoms satwa and neem guduchi (Tinospora cordifolia grew on neem) satwa were studied. Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its function. In the current development of immunotherapy use of immunomodulators has a vital place. These immunomodulators either of plant or animal origin, enhance the immune responsiveness of body against pathogens by activating the non-specific immune system. Use of medicinal plant in immunomodulation is an alternative to chemotherapy for many diseases.

Neutrophil plays an important role in host immune mechanism system. The neutrophilic phagocytic system has many advantages. Even the foreign bodies, thermal or chemical burns, bacterial infection, and other types of injuries can provoke an intense neutrophil response. The vast number of cells can be mobilized due to any set of chemotactic stimuli, moreover, neutrophils are highly effective at killing certain bacteria and their ability to digest cellular debris, and exogenous particulate matter provides an important step in the healing process.

Thus, it can be suggested that immunomodulation could be attained through increased neutrophil adhesion stimulated by neem guduchi satwa at a dose of 300 mg/kg when compared to other treated groups. Hemoglobin count in neem guduchi (300 mg/kg/p.o.) treated group showed 12.3 ± 0.543 gm% increases in Hb count compared to prednisolone treated group which was 11.9 ± 0.602 gm%, but no significant difference observed when compared to healthy control. RBC count doesn’t show any significant difference between neem guduchi and other treated group.

The hemagglutination antibody titer was used to assess humoral immune response. This test was performed to estimate serum immunoglobulin levels to evaluate the increase in serum immunoglobulin production after the administration of the drug. satwa of neem guduchi show increasing antibody titer in rat’s blood. Major immunoglobulin’s namely IgG and IgM are central...
to humoral immune responses, which are involved in the complement activation, opsonization, neutralization of toxins, etc. In neem guduchi satwa treated group antibody titer was 5.2 ± 0.74 at a dose of 300 mg/kg was found statistically significant only when compared to prednisolone standard drug used in study. Thus the result showed that action of the neem guduchi significantly increases the humoral immune response.

The DTH response was found to be increased at a dose of 300 mg/kg neem guduchi satwa followed by Tinospora cordifolia satwa treated group which was 0.29 ± 0.08 and 0.27 ± 0.04 respectively which is directly associated with cell-mediated immunity (CMI). Cell-mediated immunity involves effective mechanism carried out by T lymphocyte and their products (lymphokines). CMI responses are critical to defense against infectious organisms, infection of foreign grafts, tumor immunity, and delayed-type hypersensitivity reactions.

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. DTH requires the specific recognition of a given antigen by activated T-lymphocytes, which subsequently proliferate and release cytokines resulting in increased vascular permeability. 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 compared to other treated groups.

Different parts of medicinal plants are used for their active constituents in many ways, especially for drugs. It is owing to their high curing value and wild occurrence in diverse environments, a substantial number of literature reports on the response of medicinal plants to salinity stress. Little information is available on medicinal plants. Soil is a major influence factor involved in plant growth as well as the production of secondary metabolites, which is used as medicinal compound in a variety of many diseases. The content of some secondary plant products is significantly higher in plants grown under salt stress, availability of organic carbon, micro and macro elements in soil. The concentrations of various secondary plant products are strongly dependent on the growing conditions, especially stress conditions source of essential minerals like iron, copper, sodium, nickel, magnesium, etc.

In neem guduchi soil contain total iron 9.40 ppm, copper 5.10 ppm, organic carbon 2.22%, conductivity 0.36 ds/m followed by Tinospora cordifolia soil contain total iron 21.95 ppm, copper 12.73 ppm organic carbon 4.60%, conductivity 0.19 ds/m. Previous Vermani et al., reported 1226 ppm iron concentration for Tinospora cordifolia soil. Previously, the effect of micronutrients, such as copper and zinc on secondary metabolite production was reported by Sivakumar et al. during micropropagation study of Tinospora cordifolia. This data suggested that availability of organic carbon, micro and macro elements in the soil contributes to variation in the production of secondary metabolites, which is used as a medicinal compound in a variety of many diseases. Minimum availability of this content in neem guduchi soil is responsible for the presence of unknown but beneficially compound acts as immunomodulator or immunostimulator.

Chromatographic study (HPTLC) was carried out under the 220 nm UV to establish the fingerprint profile of Tinospora cordifolia and neem guduchi. Neem guduchi showed the presence of an additional peak at Rf 0.80. The presence of phytocomponent at Rf 0.80 value might be responsible for the immunomodulatory activity of neem guduchi.

CONCLUSION: In conclusion, the result of the present study indicated that neem guduchi have comparatively highest immunomodulatory activity than guduchi; there have been recommended to be used in guduchi preparation prescribed for immunomodulation. The unknown secondary metabolite present in neem guduchi (Rf 0.80) which may act as enhanced immunomodulator because of the presence of minimum soil nutrients variable organic carbon, available nitrogen, iron, manganese, and copper content in neem guduchi. But based on obtained results, it cannot yet be conclusively said that additional peak in neem guduchi is responsible for its optimum ratio of the
various constituents which makes it superior over the other. Finally, there is a need to know further characterization of immunomodulatory components as well as soil components. The general objective of the future will be soil studies for its better understanding of the response of production of secondary metabolites in medicinal plants.

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