IJPSR (2019), Volume 10, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 November 2018; received in revised form, 13 June 2019; accepted, 26 June 2019; published 01 July 2019

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

Rahul Sonavale [2], Arti Narkhede [1], Anuradha Mulik [1], Bipinraj Kunchiraman [2] and Suresh D. Jagtap [*1]

Interactive Research School for Health Affairs (IRSHA) [1], Bharati Vidyapeeth Deemed University, Pune - 411043, Maharashtra, India.
Rajiv Gandhi Institute of Biotechnology [2], Bharati Vidyapeeth Deemed University, Pune - 411045, Maharashtra, India.

Keywords:

Tinospora cordifolia, Neem guduchi, Satwa, Immunomodulation, Edaphic factors

Correspondence to Author:
Dr. Suresh D. Jagtap

Associate Professor,
Department of Herbal Medicine,
Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth (Deemed to be University), Pune-Satara Road, Pune - 411 043, Maharashtra, India.

E-mail: chiritatml@rediffmail.com

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 immuno-modulator. 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:

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(7).3386-94

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(7).3386-94

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 [1]. 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 [2, 3]. 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 immunoaugmentary 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 [4].

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.* [5] 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 [6].

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 [7, 8]. However, the distribution of metabolites in the root parts was mainly affected by variation in edaphic factors [9].

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 were reconstituted in water to get the desired dose [10].

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 ℃ 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 weigh 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 intra-peritoneal 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 7^{th} 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 ℃. 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:

$$\text{Neutrophil adhesion (\%)} = NI_U - NI_T / NI_U \times 100$$

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 [14].

HPTLC: Both satwa is separated and estimated quantitatively on pre-coated silica gel 60 G_{254} 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 [15].

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.

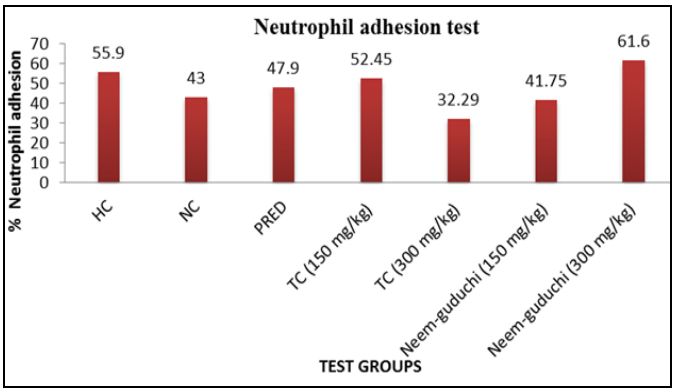


FIG. 1: EFFECT OF *TINOSPORA CORDIFOLIA* AND NEEM-GUDUCHI ON NEUTROPHIL ADHESION TEST. All values are mean ± SEM, n=7, Compared with negative control HC=Healthy control, NC= Negative control, TC=*Tinospora cordifolia* TCN=*Tinospora cordifolia* neem guduchi.

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

Groups	Treatment	Hb count (gm %)	RBC Count ($10\times^{6}$/µl)
I	HC	12.1 ±0.368	8.2 ± 0.668
II	NC	10.2 ±0.776	7.2 ± 0.441
III	Prednisolone	11.9 ± 0.602**	8.1 ±0.694
IV	TC 150 mg/kg	11.1 ±0.2	7.7 ±0.206
V	TC 300 mg/kg	11.4 ± 0.736	7.8 ± 0.330
VI	TCN 150 mg/kg	10.8 ± 0.805	7.5 ± 0.818
VII	TCN 300 mg/kg	12.3 ±0.543***	8.2 ± 0.25

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**.

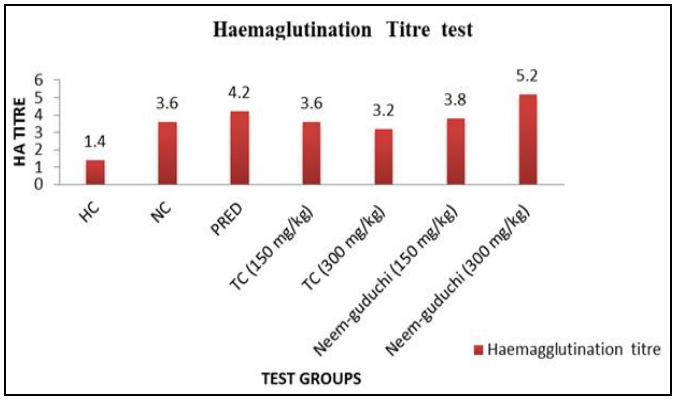


FIG. 2: EFFECT OF *TINOSPORA CORDIFOLIA* AND NEEM GUDUCHI ON HUMORAL IMMUNE RESPONSE. All values are mean ± SEM, n=7, compared with negative control, ** P<0.01, HC=Healthy control, NC= Negative control, TC=*Tinospora cordifolia*, TCN=*Tinospora cordifolia* neem guduchi.

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.

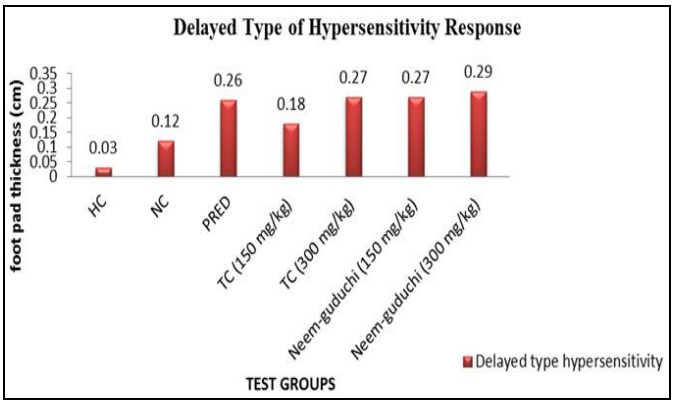


FIG. 3: EFFECT OF *T. CORDIFOLIA* AND NEEM-GUDUCHI SATWA ON DELAYED TYPE OF HYPER-SENSITIVITY RESPONSE. All values are mean ± SEM, n=7,*p<0.05 when compared with negative control HC=Healthy control, NC= Negative control, TC=*Tinospora cordifolia* TCN=*Tinospora cordifolia* neem guduchi.

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

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

All values are mean ± SEM, n=7.

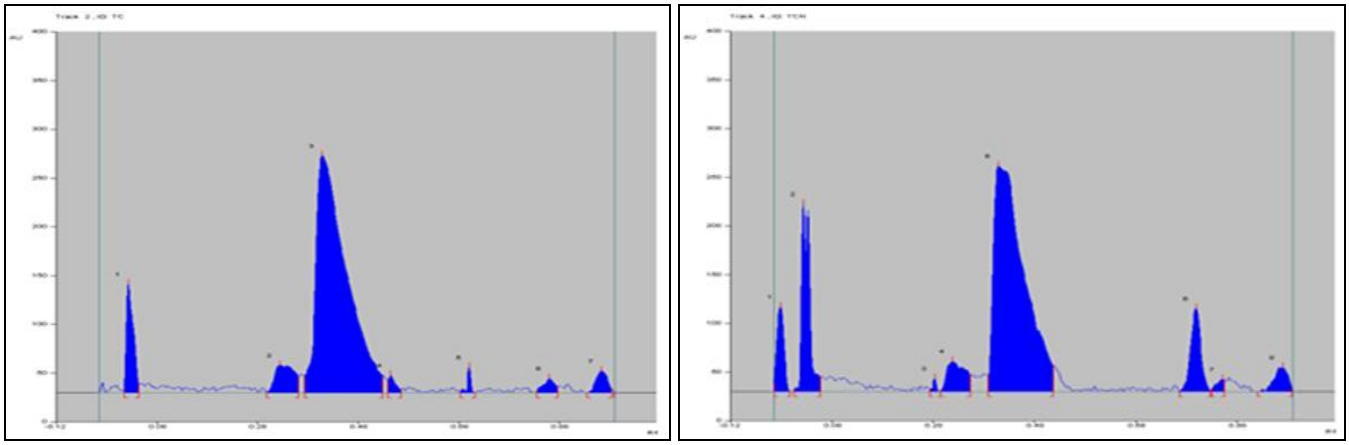
Soil Test: Following the table, **Table 3** shows the parameter studied for the soil collected from different locations. By summarizing above, the value of organic carbon in *Tinospora cordifolia* soil is 4.60 more than neem guduchi plant soil 2.22. Total iron content in *Tinospora cordifolia* plant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

Parameters	Specification	Remark	Neem Guduchi	*T. cordifolia*
pH	4.51-5.50	Acidic	6.61	6.41
	6.51-7.50	Normal pH		
	7.51-8.50	Alkaline		
Conductivity ds/m	Less than 1.00	Normal	0.36	0.19
Free lime %	Less than 0.50	Very Less	2.00	2.00
	1.01-2.00	Normal		
	2.01-5.00	More than required		
Organic carbon%	0.81-1.00	High	2.22	4.60
	More than 1.01	Very High		
Available phosphorous Kg/hector	Less than 7.00	Very less	^{17}.92	13.44
	07.01-14.00	Less		
	14.01-21.00	Normal		
Available Nitrogen	281-420	Normal	470.4	672.0
Kg/hector	421-560	More than required		
	561-700	High		
Available potassium Kg/hector	Less than 100	Very Less	672.0	560.0
	More than 301	Very high		
Calcium ppm	0501-1000	Normal	4725.0	3750.0
	1001-5000	High		
	More than 5000	Very High		
Magnesium ppm	Less than 250	Less	1325.0	1175.0
	More than 1001	Very High		
Sulphar ppm	11-50	Normal	329.0	259.0
	More than 101	Very High		
Total iron ppm	2.10-4.50	Normal	9.40	21.95
	More than 4.51	High		
Manganese ppm	1.10-2.00	Normal	17.65	24.28
	More than 2.01	High		
Zink ppm	0.51-1.00	Normal	2.15	3.38
	More than 1.01	High		
Copper ppm	0.21-1.00	Normal	5.10	12.73
	More than 1.01	High		
Sodium ppm	Less than 1000	Safe	250	210
	More than 1001	Not Safe		
Cation exchange Ratio Meq/100g	---	--	36.53	42.88
Sand %	---	--	24.0	22.20
Silt %	--	--	48.0	53.00

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) **B) HPTLC FINGERPRINT OF NEEM GUDUCHI SATWA (10 mg/ml, w/v)**

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 R*f* VALUE AND AREA UNDER THE PEAK

Guduchi			Neem Guduchi		
Peak number	R_f value	Area under the peak	Peak number	R_f value	Area under the peak
1	0.03	1131.7	1	-0.02	939.3
2	0.33	847.2	2	0.03	2255.1
3	0.41	11330.0	3	0.29	68.8
4	0.55	197.1	4	0.32	870.1
5	0.70	125.9	5	0.41	10810.7
6	-	-	6	0.80	1306.7
7	0.86	244.6	7	0.85	152.9
8	0.97	384.9	8	0.97	524.6

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 R_f 0.03, 0.33, 0.41. 0.55, 0.70, 0.86, 0.97 and 8 spot at R_f-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 R_f 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.

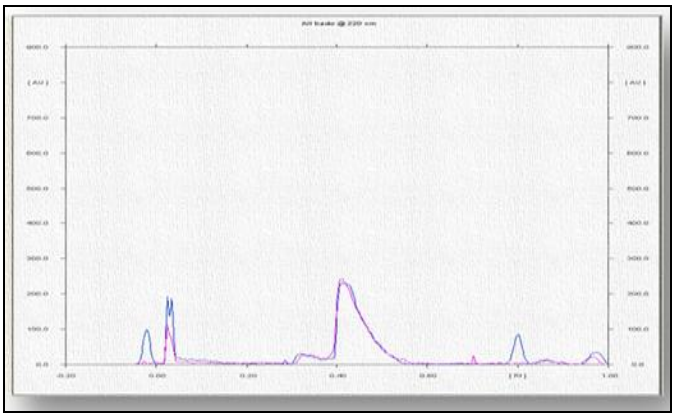


FIG. 5: HPTLC PROFILE OF GUDUCHI AND NEEM GUDUCHI SATWA IN AQUEOUS AT 220 nm. Pink-*Tinospora-cordifolia,* Blue-Neem Guduchi.

DISCUSSION: *T. cordifolia* reported to have beneficial effects on the immune system by using their aqueous and alcoholic extracts [16, 17]. 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 form any diseases [15].

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 [18].

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.* [19] 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 [20].

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 [21]. 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 [22]. 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 **Table 3**. Previously Vermani *et al.,* [23] 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.* [24] 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 R_f 0.80. The presence of phytocomponent at R_f 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 (R_f 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.

ACKNOWLEDGEMENT: Authors are thankful to the authorities of Interactive Research School for Health Affairs (IRSHA) and Bharati Vidyapeeth Deemed University for providing the essential infrastructure.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Newman DJ and Cragg GM: Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products 2016; 79: 629-61.
2. Ang HY, Subramani T, Yeap SK, Omar AR, Ho WY, Abdullah MP and Alitheen NB: Immunomodulatory effects of *Potentilla indica* and *Dendrophthoe pentandra* on mice splenocytes and thymocytes. Experimental and Therapeutic Medicine 2014; 7: 1733-37.
3. Ngcobo M, Gqaleni N, Naidoo V and Cele P: The immune effects of an african traditional energy tonic in *in-vitro* and *in-vivo* models. Evidence-Based Complementary and Alternative Medicine 2017; 1-14.
4. More P and Pai K: Immunomodulatory effects of *Tinospora cordifolia* (Guduchi) on macrophage activation. Biology and Medicine 2011; 3(2): 134-140.
5. Upadhyay AK, Kumar A, Om H and Mishra S: *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi)- Validation of the Ayurvedic pharmacology through experimental and clinical studies. International Journal of Ayurveda Research 2010; 1(2): 112-20.
6. Gowrishankar R, Kumar M, Menon V, Divi SM and Saravanan M: Trace element studies on *T. cordifolia* (Menispermaceae), *O. sanctum* (Lamiaceae), *M. oleifera* (Moringaceae), and *P. niruri* (Euphorbiaceae) using PIXE. Biological Trace Element Research 2010; 133: 357-63.
7. Sharma R, Hetal A, Galib and Prajapati PK: Antidiabetic claims of *Tinospora cordifolia* (Willd.) Miers: critical appraisal and role in therapy. Asian Pacific Journal of Tropical Biomedicine 2015; 5(1): 68-78.
8. Mangal M, Sheoryan A, Mangal AK, Kajla S, Choudhury A and Dhawan A: Biotechnological advances in *Tinospora cordifolia* (Willd.) Miers Ex Hook. F. & Thoms: Overview of present status and future prospects. International Journal of Plant Research 2012; 25(2): 182-91.
9. Sampaio BL, Edrada-Ebel R and Costa FB: Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants. Scientific Reports 2016; 6: 29265.
10. Patel S, David B, Otilia J, Banji F, Patel MM and Shah KK: Scrutinizing the role of aqueous extract of Trapa bispinosa as an immunomodulator in experimental animals. International Journal of Research and Pharmaceutical Sciences 2010; 1: 13-19.
11. Ismail S and Asad M: Immunomodulatory activity of *Acacia catechu*. Indian Journal of Physiological Pharmacology 2009; 53(1): 25-33.
12. Bhalerao BM, Kasote D, Nagarkar B, Jagtap S, Vishwakarma K, Pawar P and Maheshwari V: Comparative analysis of radical scavenging and immunomodulatory activities of *Tinospora cordifolia* growing with different supporting trees. Acta Biologica Szegediensis 2012; 56(1): 65-71.
13. Zamani Z, Zeinali H and Madani J: Effect of nitrogen and phosphorous fertilizers on the yield and secondary metabolites of medicinal plant *Rubia tinctorum* L. under salinity conditions. Iran J of Plant Phy 2014; 4(2): 949-55.
14. Puratchimani V and Jha S: HPTLC standardization of Tinospora cordifolia using tinosporaside. Indian Journal of Pharmaceutical Sciences 2007; 69: 578-81.
15. Adhvaryu MR, Reddy N and Vakharia BC: Prevention of hepatotoxicity due to anti tuberculosis treatment: A novel integrative approach. World Journal of Gastroenterology 2008; 14: 4753-62.
16. Panchabhai TS, Ambarkhane SV, Joshi AS, Samant BD and Rege NN: Protective effect of *Tinospora cordifolia*, *P. emblica* and their combination against antitubercular drugs induced hepatic damage: An experimental study. Phyto-therapy Research 2008; 22: 646-50.
17. Singh M, Tailang M and Mehta SC: A review on herbal plants as Immunomodulators. International Journal of Pharmaceutical Sciences & Research 2016; 7(9): 3602-10.
18. Yadav R, Kharya DM, Yadav N and Savadi R: Immunomodulatory potential of ethanol extract of *Spilanthus acmella* leaves. International Journal of Biological & Medical Research 2011; 2(3): 631-35.
19. Gaur K, Kori ML and Nema RK: Comparative screening of immunomodulatory activity of hydro-alcoholic Extract of *Hibiscus rosasinensis* Linn. and ethanolic extract of *Cleome gynandra* Linn). Global Journal of Pharmacology 2009; 3(2): 85-89.
20. Sharma R, Amin H, Galib R and Prajapati PK: Seasonal variation on the physicochemical profile of guduchi satwa (a starchy substance from *T. cordifolia* [Willd] Miers). J of Ayurveda Integration Medicine 2013; 4: 193-97.
21. Dashputre NL and Naikwade NS: Immunomodulatory activity of *Abutilon indicum* Linn. on albino mice. International Journal of Pharma Sciences and Research 2010; 3: 178-84.
22. Nikolova MT and Ivancheva SV: Quantitative flavonoid variations of *A. vulgaris* L. and *V. chamaedrys* L. in relation to altitude and polluted environment. Acta Biologica Szegediensis 2005; 49: 29-32.
23. Vermani A, Navneet, Prabhat and Chauhan A: Physico-chemical analysis of ash of some medicinal plants growing in Uttarakhand, India. Nature & Science 2010; 8(6): 88-91.
24. Sivakumar V, Rajan MSD, Sadiq AM and Jayanthi M: *In-vitro* micropropagation of *Tinospora cordifolia* (Willd.) Miers Ex Hook. F. & Thoms - An important medicinal plant. Journal of Pharmacognosy and Phytochemistry 2003; 3 (2): 5-10.

How to cite this article:
Sonavale R, Narkhede A, Mulik A, Kunchiraman B and Jagtap SD: Effect of edaphic factors on major secondary metabolites of *Tinospora cordifolia* and neem guduchi with respect to their immunomodulatory effect. Int J Pharm Sci & Res 2019; 10(7): 3386-94. doi: 10.13040/IJPSR.0975-8232.10(7).3386-94.