wjpmr, 2020,6(5), 22-28

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

IMMUNOMODULATORY EFFICIENCY OF *TINOSPORA CORDIFOLIA* AGAINST VIRAL INFECTIONS

***[1]K. G. C. Dissanayake, [2]W. P. R. T. Perera and [3]N. Premasinghe**

[1]Department of Cikitsa, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Yakkala, Sri Lanka.
[2]Research and Publication Division, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Yakkala, Sri Lanka.
[3]Department of Chemistry, University of Kelaniya, Dalugama, Kelaniya, Sri Lanka.

***Corresponding Author: K. G. C. Dissanayake**

Department of Cikitsa, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Yakkala, Sri Lanka.

Article Received on 03/03/2020 **Article Revised on 24/03/2020** **Article Accepted on 14/04/2020**

ABSTRACT

Most of the cases, viral infected patients are suffering from secondary infections. Apart from that, due to the exhausted immune system patients more vulnerable to secondary infections and various diseases. Dysfunction of the immune system is responsible for multiple illnesses, such as arthritis, ulcerative colitis, asthma, allergies, parasites, cancer, and infectious diseases. So, medicinal plants and their active components are becoming increasingly relevant as a source of immunomodulatory agents. *T. cordifolia* stem extracts or the isolated compounds of the plant exhibits amazing immune stimulatory effects in various ways. Isolated compounds of *T. cordifolia* such as N-methyl-2-pyrrolidone and 11-hydroxymustakone, Magnoflorine and Tinocordiside shows immunomodulatory effects by enhancing Reactive Oxygen Species (ROS) generation which causes to augment the immune response. *T. cordifolia* extract exhibits a considerable effect of the immunostimulation in HIV positive patients and, increases the phagocytosis and intercellular killing capacity by increasing the survival rate and polymorphonuclear leucocyte function. In addition to that, a novel (1,4)-α-D-glucan from *T. Cordifolia* activates the immune system by activating macrophages via of TLR6 signaling and NF-κB activation mechanism, leading to cytokine and chemokine production. Immunoductatory protein (ImP) obtained from the dry stem powder of *Tinospora cordifolia* is significant for augmenting the various immunological activities in the human body. Hence, more attention should be focused on the phytochemistry and their applications of the *Tinospora cordifolia* for immune enhancements as well as reduce secondary infections risks along with the viral infections.

KEYWORDS: Tinospora cordifolia, Immunomodulatory, Viral infections, Phytochemistry.

1. INTRODUCTION

Plants are used in both structured (Ayurveda, Unani) & Unorganized (folk, tribal, native) ways as therapeutic agents since time immemorial. In both the developing and developed countries, the demand for medicinal plants is increasing. Medicinal plant science is one of the leading research areas globally. Any of these medicines are believed to promote good health and preserve organic resistance against infection by restoring body balance and conditioning the body tissues. Meanwhile, a valuable plant was found as a perfect immune-stimulatory herb called *Tinospora cordifolia.* Apart from the normal anti-bacterial, anti-viral, anti-cancer, properties, and various health benefits, *T. cordifolia* exhibits immune-stimulatory efficiency by augmenting macrophage chemotaxis, phagocytosis and promotes interaction with other immune-regulatory lymphoid cells.[1]

A summary of morphological features of the *Tinospora cordifolia* can be described as Flowers are unisexual, axillary, leaflet branches 2–9 cm long and greenish-yellow in color, male flowers are clustered, the female is usually solitary. The fruits are single-seeded, the winter fruits and the summer flowers. The root is thread-like, aerial, often constantly extending the touch of the ground. And aerial roots are characterized by tetra to the primary structure of the pent arch. The seeds are in a curved form and the endocarp is ornamented in various ways.[2,3] *T. cordifolia* (synonym: Tinospora sinensis) is also known as Guduchi/Amrita in India and its names in Latin: Tinospora cordifolia (Wild), English: Tinospora /Indian Tinospora, Hindi: Giloya, Sanskrit: Amritha, and Sinhala: Rasakinda. It belongs to the family of Menispermaceae and is found in Myanmar, Sri Lanka, and China.[4]

Taxonomical Classification[5]
Kingdom: Plantae
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Ranunculidae
Order: Ranunuculales
Family: Menispermaceae
Genus: Tinospora
Species: Cordifolia

Medicinal significance of *Tinospora cordifolia*

There are so many amazed and valuable Ayurveda medicinal properties that can be found out in *Tinospora cordifolia* stem and bark. The *Tinospora cordifolia* stem is one of the components of Ayurveda preparations used in general debility, dyspepsia, cough, and urinary diseases. The seed is salty, diuretic and stomachic Stimulates bile secretion, induces constipation, soothes hunger, burning pain, diarrhea, blood enrichment, and jaundice cures.[6,7] Roots and stem extract of the *Tinospora cordifolia* use as an antidote to the snake bite and scoping sting combining with other drugs.[8,9,10] Dry bark of *Tinospora cordifolia* has antipyretic effects.[11], Anti-allergicc and anti-inflammatory effects.[12,13] The stem's aqueous extract antagonizes the effect of the agonists such as 5-hydroxytryptamine, histamine, bradykinin, and prostaglandins E1 and E2 on rabbit smooth muscle. And relaxes the intestinal, uterine smooth muscle and inhibits the constrictor reaction of histamine and acetylcholine to smooth muscle.[14] The *T. cordifolia* is commonly used in Ayurvedic Indian medicine to treat diabetes mellitus. Oral regulation of aqueous *T. Cordifolia* root extract to alloxan diabetic rats caused substantial blood glucose and brain lipid reduction.[15] Another important phenomenon is the hepatoprotective ability of *T. cordifolia* extracts. In an experiment, goats treated with *T. cordifolia* have shown significant clinical and hemato-biochemical improvement in CCl_4 induced hepatopathy. In vitro, cordifolia also displayed inactivating properties against surface antigen Hepatitis B and E.[16] *T. cordifolia* Dried Stem has had a major anti-inflammatory effect in both acute and subacute inflammatory models. The *cordifolia* was found to be more effective in acute inflammation than acetylsalicylic acid as well.[17] Apart from the above-mentioned pharmacological effects, the aqueous extract of roots of *T. cordifolia* has shown the potential of anti-oxidant action in alloxan diabetes rats. The administration of the extract of *T. cordifolia* roots (2.5, 50 mg/kg body weight) for 6 weeks resulted in a significant reduction of serum and tissue cholesterol, phospholipids and free fatty acids in alloxan diabetic rats.[18]

This plant's pharmacological activities are attributable to its chemical components, such as diterpenoid lactones, glycosides, hormones, sesquiterpenoids, phenolic, aliphatic compounds, essential oils, a mixture of fatty acids which polysaccharides which are found in a different part of the plant's body, like roots, stem, and leaves. The chemical elements of *T. Cordifolia* belong to several groups such as alkaloids, glycosides, hormones, phenolic, aliphatic compounds, polysaccharides, protein-rich leaves, calcium, and phosphorus. The stem contains chlordane furono diterpene glucoside (Amrithocides).[19]

Impact of viral infections to the immune system

Phagocytosis is an effective immune system mechanism used to remove pathogens, foreign particles, and cell debris in the body.[20] Phagocytosis is a multistep process that begins with the use of surface receptors to identify the target. Recognition is accompanied by membrane protrusion to form a phagocytic cup around the target and to form a phagosome into the cell. Phagolysosomes contain lytic enzymes and chemicals such as peroxidase, lysozyme, hydrolyze enzyme and hydrogen peroxide that cause oxidative bursts to digest its contents. The last stage of phagocytosis is removal by exocytosis of the phagolysosome contents.[21] Phagocytosis in mammals is primarily performed by macrophages, neutrophils, monocytes, dendritic cells, and mast cells, also referred to as professional phagocytes.[22] Phagocytes and their phagocytosis potential are an integral part of the innate immune system and are essential to the host's homeostasis. Impairment in phagocytosis was related to various diseases and disorders. The phagocytic cycle has been shown to affect different cytokines. Cytokines including TNFα, IL-1β, GM-CSF, and TGF-β1 have been found to promote phagocytosis.[23] Elements of the cellular immune system also play key roles in virus response by the host. These elements can be classified into two groups. Innate immune cells (macrophages, granulocytes, and NK cells) and adaptive immune cells ('helper' TCD4 + cells and cytotoxic TCD8 + cells).[24]

Once a certain virus infects host cells, the immune system of the human body will be negatively affected in several ways. First, the replication of viruses in the cells of the immune system may affect the immune system's functional ability and thus lead to immunological disturbances. Second, the host's immune response to viral antigens can lead to the formation of circulating virus-antibody complexes. In addition to that, the interaction of sensitized lymphocytes or antiviral antibodies and the complement of virus-induced cell surface antigens may lead to cell injury.[25,26] Viruses have evolved strategies to evade the inflammatory and immune responses in their hosts. There are so many strategies are followed by viruses to manipulate the immune system. Especially, the human immunodeficiency virus (HIV), which is highly successful in modifying the immune system despite its smaller genome. Most of the viruses cause to neutralize chemokines by secreting the binding proteins to disrupt the immune response. That means Viruses can modulate the chemokine network by encoding chemokine homologs (vCKs) or chemokine receptors (vCKRs) or by secreting chemokine-binding proteins (vCKBPs) which have no sequence similarity with host proteins.[27]

The main motive of this review is to understand the immunology of the human body and its' impact on the viral infections and reveals the abilities to utilize the phytochemistry of the *Tinospora cordifolia* against viral infections as well as for immuno-boosting. And persuasion to use to do mere work with natural plant materials for medicines regarding viral infections and its' secondary effects.

2. METHODOLOGY

Literature searches were performed using the words " *Tinospora cordifolia* " or "Amrutha" or "Guduchi in PubMed, PMC and Science direct academic Search Complete. These words have also been typed into popular search engines, including Google and Google Scholar, to discover secondary details and items containing this herb available for purchase through the Internet. All material, regardless of source, was reviewed, and the review framework was developed to represent the information available. Chemical structures of the phytochemicals were drawn using "MedChem designer" software.

3. RESULTS AND DISCUSSION

Immunomodulatory actions

Dysfunction of the immune system is responsible for multiple illnesses, such as arthritis, ulcerative colitis, asthma, allergies, parasites, cancer, and infectious diseases.[28] Currently, chemotherapeutic agents are primarily immunosuppressive, although most of them are cytotoxic and have a range of side effects. Therefore, medicinal plants and their active components are becoming increasingly relevant as a source of immunomodulatory agents. Several researchers have well reported the use of plant products to enhance the phagocytic ability of macrophages and increase the development of antibodies by B cells.[29]

The main significant Isolated compounds of the *Tinospora cordifolia* are N-formylannonain,[30] Cordifolioside,[31] Magnoflorine,[32] Tinocordiside,[33] Syringin, N-methyl-2-pyrrolidone and 11-Hydroxymuskatone.[34] (Fig. 1)

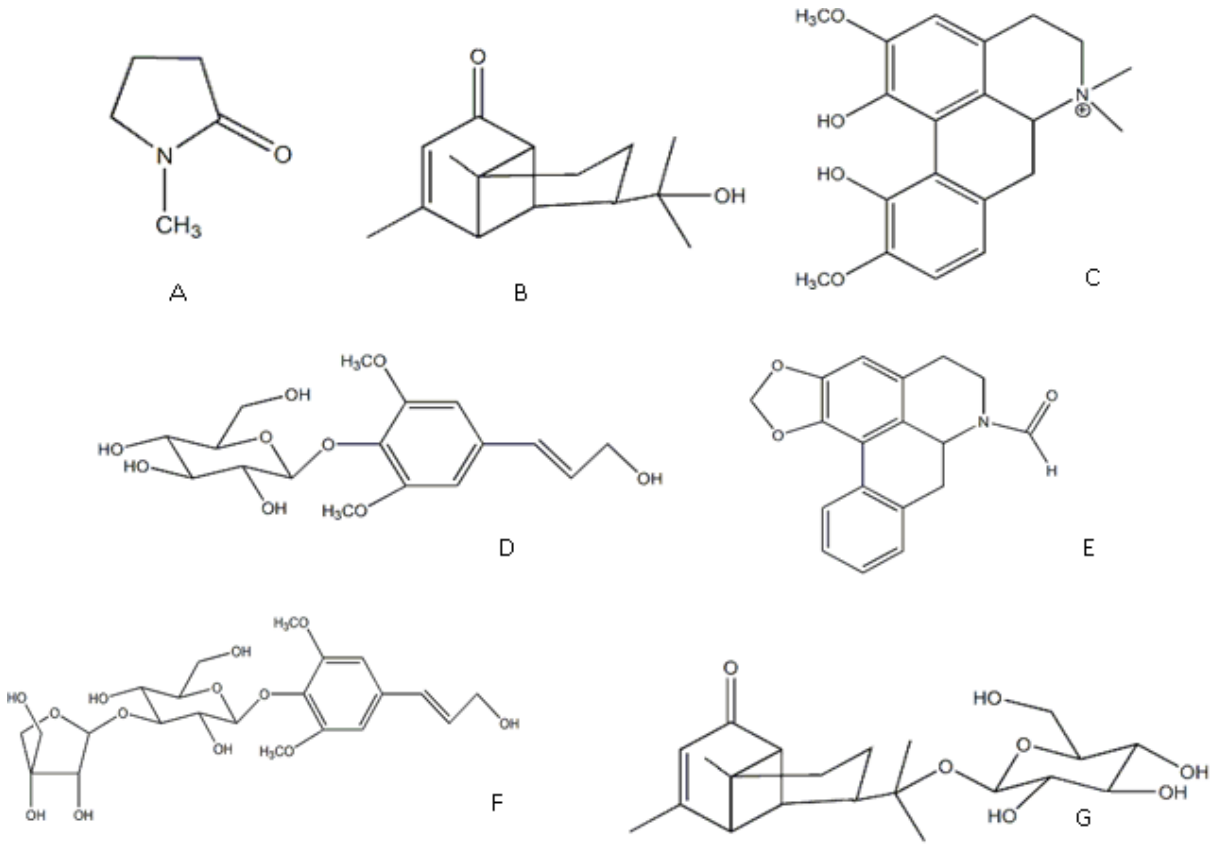

Figure 1: Major isolated compounds in stem extract and amorphous powder of the *Tinospora cordifolia;* (A) N - Methyl-2-pyrrolidone, (B) 11-Hydroxymustakone, (C) Magnoflorine, (D) Syringin, (E) N-Formylannonain , (F) Cordifolioside A, (G) Tinocordiside.

According to the studies of Sharma (2012) the immunomodulatory role of the various fractions and stem extracts of *Tinospora cordifolia* has been evaluated using phagocytic function studies of polymorph nuclear neutrophil (PMN). Chromatographic purification of the active fractions led to the isolation of seven compounds. Using the phagocytic function test PMN, the immunomodulatory capacity of isolated compounds was also estimated. The isolated compounds were estimated to activate the ROS *in vitro* using three separate assays,

viz. Assays of nitro blue tetrazolium (NBT), nitrous oxide (NO), and chemiluminescence.[35] Reactive oxygen species (ROS) are a group of highly reactive chemicals, comprising either exogenously or endogenously derived oxygen. ROS is associated with a wide variety of human illnesses, including chronic inflammation, age-related diseases, and cancers. ROS is also important for diverse biological functions, including cell survival, cell development, proliferation and differentiation, and immune response.[36] professional phagocytes in humans (mainly neutrophils and macrophages) utilized a membrane-bound NADPH oxidase to generate a large amount of O_2^{2-}, which was then dismutase to H_2O_2 and oxygen by SOD. This large production of ROS is the main weapon of phagocytes to fight against invading microorganisms, a process known as "respiratory burst" or "oxidative burst." Since then, studies on the role of ROS and antioxidant defense systems under physiological and pathophysiological conditions have had a great impact on the field of immunology.[37]

To evaluate Reactive Oxygen Species (ROS) generation via the superoxide formation process three types of Assays have been carried out. Nitro Blue Tetrazolium (NBT) assay reveals that the Isolated compound's fraction of ethyl acetate of *Tinospora cordifolia* was evaluated on a concentration scale of 0.1–50 g / ml. All concentrations showed an increase in crystal formation suggesting superoxide generation as compared to control. The maximum effect was observed at 0.5 and 1 g / ml, however, with a dose-dependent decrease in activity at higher concentrations. A concentration range of 0.01–2.5 g / ml was evaluated for the mixture of N-methyl-2-pyrrolidone and 11-hydroxymustakone, Magnoflorine and Tinocordiside. All concentrations of tested molecules showed an increase in formazan crystal formation suggesting superoxide generation compared with control. Ethyl acetate fraction was evaluated over a concentration range of 0.1–50 g/ml in the Nitric oxide (NO) assay and more or less the same results observed. According to the chemiluminescence assay, All ethyl acetate fraction concentrations showed a time-dependent luminescence increase indicating increased ROS generation compared to control. N-methyl-2-pyrrolidone and 11-hydroxymustakone treated cells showed a dose-dependent increase in luminescence, suggesting an increased ROS generation.[35]

Immunostimulant for HIV positive patients

Acquired immunodeficiency syndrome AIDS is a fatal illness caused by Human Immuno-Deficiency Virus (HIV), which breaks down the host immune system., leaving the subject vulnerable life-threating opportunistic infections and neurological diseases.[38] Thymus-derived-lymphocytes deficiency is the main defect of the HIV viral infections.[39] Owing to the absence of an effective cure, immunorestorative therapy will be in a negative stage.[40]

According to a study performed using an HIV positive volunteer group reveals the considerable effect of the immunostimulation in the *Tinospora cordifolia* extract (TCE). The TCE treated group showed a significant reduction of Leukocyte and it may be due to its "Rasayana" effect. In addition to that TCE treated group showed a reduction of Eosinophil count compare to the control supporting the immunomodulatory effect of the stem extract of the *Tinospora cordifolia*,[41] While another study reveals that *Tinospora cordifolia* extract shows effects against *E. coli* induced peritonitis in a mice study. It has found significant improvement of Bacterial clearance, as well as improved phagocytic and intracellular bacterial capacities of neutrophils in the Tinospora cordifolia extract, treated group of mice. The plant extract-treated group shows specific immunomodulatory effects than control.[42] In addition to that, Immunomodulators such as *Tinospora cordifolia* minimize the risk involved and boost the outcome in obstructive jaundice after surgery, and should be included in the jaundiced patients ' preoperative care. Immunomodulation is the need of the hour and to prevent sepsis and immunosuppression. When TEC was added o routine surgical procedure in patients with obstructive jaundiced patients, increase the phagocytosis and intercellular killing capacity by increasing the survival rate and polymorphonuclear leucocyte function.[43] Polymorph nuclear leukocytes (PMNs) are white blood cells derived from the bone marrow which play a key role in the host's defense against infection. PMNs are attracted to invading microorganisms through interactions with antibody, complement, and chemotactic factors, and are induced to phagocytose them.[44]

Allergic rhinitis

Meanwhile, Another study has discovered an efficacy of the extract of *Tinospora cordifolia* (TC) for allergic rhinitis. They were tested clinically, and DLC (desloratadine citrate disodium injection), and nasal smear were performed with Hemoglobin levels. With TC treatment 100% relief was reported from sneezing in 83% patients, 69% from nasal discharge, 61% from nasal obstruction and 71% from nasal pruritus. These results show significantly more efficient than the control (placebo). After treatment of TC, eosinophil and neutrophil count decreased and goblet cells were absent in nasal smear. Finally, it says TC significantly decreased all symptoms of allergic rhinitis.[45] Rhinitis is commonly called nasal mucosal inflammation. It is a widespread condition affecting as much as 40% of the population.[46]

Another group reported that the extract of *Tinospora cordifolia* can stimulate the B lymphocytes, macrophages, and polymorphonuclear lymphocytes.[47]

Activation of Microphage

The primary line of host defense mechanism against microbial invasion is innate immunity mediated by macrophages, neutrophils, and natural killer (NK) cells.

The innate immune system targets pathogen-associated molecular patterns (PAMPs) that are structurally retained by unique encoded receptors called pattern recognition receptors. (PRRs)[48] If the microorganism develops a resistance to the anti-biotic, the immune system will be weak while there are so many natural and synthetic immune stimulants. β-glucans are effective stimulators of the innate immune system in vertebrates and they are strong activators of the complementary system in mammals as well.[49] Such polymers have therapeutic potential because of their immune system effects, which can include anti-tumor and anti-infection activities, as well as defense against fungal, bacterial, viral and protozoan infections.[50]

Soluble and particulate β-glucans interact with cognate receptors on macrophages stimulating cytokines, chemokines, and intermediate reactive oxygen synthesis.[51] Meanwhile some researchers have focused their attention on the plant-derived α-glucans also as the immunostimulatory compounds. In this research, some of the modified (1,3)-α-D-glucan derivatives also showed powerful immunostimulatory effects on lymphocyte proliferation and antibody development.[52] A novel compound isolated from the *Tinospora cordifolia* called (1,4)-α-D-glucan (Fig. 2) shows critical immunostimulating properties. According to the studies from the Nair group 2006 a novel (1,4)-α-D-glucan from *T. Cordifolia* activates the immune system by activating macrophages via TLR6 signaling and NF-κB activation mechanism, leading to cytokine and chemokine production.[53] Investigation of immunoductatory protein (Imp) of the *Tinospora cordifolia* is significant for further research approaches. One of the research studies has focused on this phenomenon and ImP has purified by dry stem powder of the *Tinospora cordifolia* and characterization also have done with periodic acid–Schiff staining, HPLC and immunochemical analysis. The immunostimulatory activity was assessed lymphocyte proliferation and microphage activation assays. ImP was a reason to enhance the mitogenic activity effectively compare with the control. Purified ImP also induced nitric oxide production from microphage present in the isolated murine peritoneal exudates cells as well as enhanced phagocytosis of the yeast cells by microphage. So the confirmation of immunoductatory protein in stem powder of *Tinospora cordifolia* opens a pathway to more immunological investigations.[54]

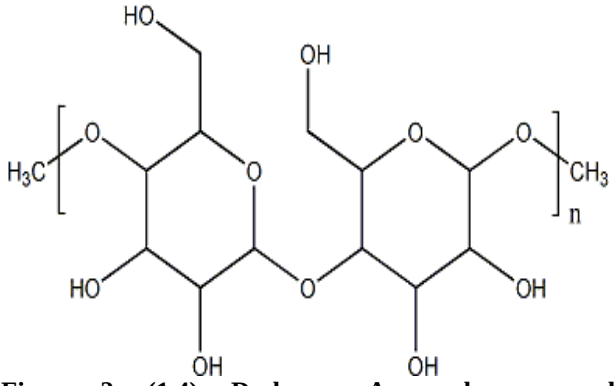

Figure 2: (1,4)-α-D-glucan; A novel compound isolated from *Tinospora cordifolia.*

Purified components of the *T. cordifolia* such as cardioids, cordiofoliosides and cordial can directly activate some functions of microphages. Even there are so many researches that have proved *T. cordifolia* act as an effective immunoductatory effect, it is worth investigating immunoductatory actions of the plant remains in a tumor-bearing host where the immune system is in a highly suppressed state. Immunosuppressive actions have been reported on the growth of DL (Dolton lymphoma)[55] To reverse the immunosuppressive activities of the tumor-bearing host alcoholic extract of the *T. cordifolia* was used and have positive results. According to the study of the studies of Singh 2003, DL bearing mice on in vivo administration of *T. cordifolia* showed prolongation of life span and complete regression of the tumor in tumor bearing mice.[56] This reverse phenomenon of the observed DL growth may be due to two reasons. Either *T. cordifolia* can directly kill tumor cells or it activates microphages that may cause enhancement of tumor cell death.[57] Apart from that *T. cordifolia* could restore the cytokine hemostasis in the DL bearing host, which is altered along with DL growth. Moreover, DL associated regression of thymus and spleen enlargement could be reversed by *in vivo* administration of *T. cordifolia.* It has proven that *T. cordifolia* treatments support to enhancement of cytokine such as LK-1 production which is mitogenic for lymphocytes.[58]

4. CONCLUSIONS

Despite having HIV (Human Immunodeficient Virus) as the most recognized immunosuppress virus, other RNA based viruses such as SARS, MERS, and COVID- 19 like coronaviruses also disruptively affect to the human immune system by opening new ways to secondary infections. Even it is too difficult to find new medicine for the viral infections to inhibit the virus directly, proceed with the reduction of immunosuppressant procedure and confusions are most significant. Hence photochemistry of the *T. cordifolia* should be highly focused on medicine. Due to having amazing immunomodulatory actions, microphage activations and immunostimulation effects against viral infections, *T. cordifolia* stem extracts or the isolated compounds need

to be used as modern research of drug development as well as Ayurveda treatments.

REFERENCES

1. Kapil A, Sharma S. (Immunopotentiating compounds from *Tinospora cordifolia*). J Ethnopharm, 1997; 58(2): 89-95.
2. Spandana U, Ali SL, Nirmala T, Santhi M, Babu SDS. (A review on *Tinospora cordifolia*). Int J Current Pharmaceu Res, 2013; 4(2): 61–68.
3. Sinha A, Sharma HP. (Micropropagation and phytochemical screening of *Tinospora cordifolia* (Wild.) Miers Ex Hook F & Thoms: a medicinal plant). Int J Adv Pharm Biol Chem, 2015; 4(1): 114–121.
4. Saha S, Ghosh S. (*Tinospora cordifolia*: one plant, many roles). Anc Sci Life, 2012; 31(4): 151–159.
5. Nayampalli SS, Ainapure SS, Samant BD, Kudtarkar RG, Desai NK, Gupta KC. (A comparative study of diuretic effects of *Tinospora cordifolia* and hydrochloro-thiazide in rats and a preliminary phase I study in human volunteers). J Postgrad Med, 1988; 34(4): 233-236.
6. Aiyer KN, Kolammal M. Pharmacognosy of Ayurvedic Drugs, Series 1. 1st ed., Trivendram; The Central Research Institute: 1963.
7. Raghunathan K, Mittra R. Pharmacognosy of Indigenous Drugs. New Delhi; Central Council for Research in Ayurveda & Siddha: 1982.
8. Anonymous. (The Wealth of India: A Dictionary of Indian Raw materials and Industrial Products (Industrial Products-Part I)). Ind Med Gaz, 1949; 84(10): 476-477.
9. Nadkarni KM. Indian Materia Medica Vol 1. In: Nadkarni AK (eds.). 3rd ed., Mumbai; M/S Popular Prakasan Pvt. Ltd: 1976.
10. Kirtikar KR. Indian Medicinal Plants, Vol 1. In: Basu BD (eds.). 2nd ed., Dehra Dun: M/S Bishen Singh, Mahendra Pal Singh: 1975.
11. Ikram M, Khattak SG, Gilani SN. (Antipyretic studies on some indigenous Pakistani medicinal plants II). J Ethnopharmacol, 1987; 19(2):185-192.
12. Nayampalli SS, Desai NK, Ainapure SS. (Anti-allergic properties of *Tinospora cardifolia* in animal models). Indian J Pharm, 1986; 18(4): 250-252.
13. Rai M, Gupta SS. (The deposition of the secondary salts over the five pellets in rats was inhibited by the aqueous extract of *T. cordifolia*). J Res Ind Med 1966; 10:113-116.
14. Anonymous. Wealth of India: Raw materials, Vol X. New Delhi; CSIR: 1976.
15. Dhaliwal KS. Method and composition for treatment of diabetes. US Patent, US 5886029, 1999.
16. Mehrotra R, Katiyar CK, Gupta AP. Hepatoprotective compositions and composition for treatment of conditions related to hepatitis B and E infection. US Patent, US 749296, 2000.
17. Jana U, Chattopadhyay RN, Shw BP. (Preliminary studies on anti-inflammaory activity of *Zingiber officinale* Rosc., *Vitex negundo* Linn. and *Tinospora cordifolia* (wild) Miers in albino rats). Indian J Pharm, 1999; 31(3):232-233.
18. Stanely M, Prince P, Menon VP, Gunasekaran G. Hypolipidaemic action of *Tinospora cordifolia* roots in alloxan diabetic rats). J Ethnopharmacol, 1999; 64(1): 53-57.
19. Khan MM, Dul-Haque MS, Chowdhury MS. (Medicinal use of the unique plant *Tinospora cordifolia*: evidence from the traditional medicine and recent research). Asian J Med Biol Res, 2016; 2(4): 508–512.
20. Underhill DM, Goodridge HS. (Information processing during phagocytosis). Nat Rev Immunol, 2012; 12(7): 492–502.
21. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, Sadoul R, Rondeau C, Desjardins M. (The phagosome proteome insight into phagosome functions). J Cell Biol, 2001, 152(1):165–180.
22. Rabinovitch M. (Professional and nonprofessional phagocytes: an introduction). Trends Cell Biol, 1995; 5(3): 85–87.
23. Sharma L, Wu W, Dholakiya SL, Gorasiya S, Wu J, Sitapara R, Patel V, Wang M, Zur M, Reddy S, Siegelaub N, Bamba K, Barile FA, Mantell LL. (Assessment of Phagocytic Activity of Cultured Macrophages Using Fluorescence Microscopy and Flow Cytometry). Meth Mol Biol, 2014; 1172: 137-145.
24. Xu XN, Screaton GR, McMichael AJ. (Virus infections: escape, resistance and counterattack). Immunity, 2001; 15(6): 867–870.
25. Notkins AL, Mergenhagen SE, Howard RJ. (Effect of virus infections on the function of the immune system). Annu Rev Microbiol, 1970; 24: 525-538.
26. Oldstone MBA, Dixon FJ. (Immune Complex disease in chronic viral infections). J Exp Med, 1971; 134: 32-40.
27. Antonio A, Peter G, Yewdell JW. (Viruses in control of the immune system). EMBO Rep, 2002; 3(10): 927-932.
28. Shivaprasad HN, Kharya MD, Rana AC, Mohan S. (Preliminary Immunomodulatory Activities of the Aqueous Extract of *Terminalia chebula*). Pharma Biol, 2008; 44: 32-34.
29. Chopra RN, Chopra LC, Handa KD, Kapur LD. Indigenous Drugs of India. 2nd ed., Kolkata; M/S Dhar VN and Sons: 1982.
30. Maurya R, Manhas LR, Gupta P, Mishra PK, Singh G, Yadav PP. (Amritosides A , B , C and D: clerodane furano diterpene glucosides from *Tinospora cordifolia*). Phytochem, 2004; 65(14): 2051-2055.
31. Maurya R, Wazir V, Kapil A, Kapil RS. (Cordifolisides A and B, two new phenylpropenedisaccharides from Tinospora cordifoliapossessing immunostimulant activity). Nat Prod Let, 1996; 8(1): 7–10.
32. Barbosa-Filho JM, Vasconcelos THC, Alencar AA, Batista LM, Oliveira RAG, Guedes DN, Falcão HDS, Moura MD, Diniz MFFM, Modesto-filho J.

(Plants and their active constituents from South, Central, and North America with hypoglycemic activity). Braz J Pharmacog, 2005; 15 (4): 392-413.
33. Ghosal S, Vishwakarma RA. (Tinocordiside, a New Rearranged Cadinane Sesquiterpene Glycoside from *Tinospora cordifolia*). J Nat Prod, 1997; 60(8): 839-841.
34. Sipahimalani A, Norr H, Wagner H. (Phenylpropanoid glycosides and tetrahydrofurofuranlignan glycosides from the adaptogenic plant drugs *Tinospora cordifolia* and *Drypetes roxburghli*). Planta Med, 1994; 60 (6): 596–597.
35. Sharma U, Bala M, Kumar N, Singh B, Munshi RK. (Immunomodulatory active compounds from *Tinospora cordifolia*). J Ethnopharmacol, 2012; 141(3): 918-926.
36. Yang Y, Bazhin AV, Werner J, Karakhanova S. (Reactive Oxygen Species in the Immune System). Int Rev Immunol, 2013; 32(3): 249-270.
37. Babior BM, Kipnes RS, Curnutte JT. (Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent). J Clin Invest, 1973; 52(3):741–744.
38. Park, K. Preventive medicine in obstest peadiatrics and Geriatrics. In: Park's text book on preventive and social medicine, 24 th ed. Jabalpur: M/S Banarasidas Bhanot Publishers, 2017.
39. Fauci AS, Braunwald E, Kasper DL, Wilson JD, Isselbachler KJ, Martin JB, Longo DL, Hauser SL, Harrison TR, Fauci A, Tapsel FA. Harrison's principles of internal medicine companion Hand book, 14th ed. London; Mcgraw-Hill: 1999.
40. Kalra S, Wadhwa P, Kalra N. (Rhinitis: Recent advances). Hosp Today, 2002; 7: 461-463.
41. Kalika MV, Thawani VR, Varadpanda UK, Sontakke SD, Singh RP, Khiyani RK. (Immunomodulatory effect of *Tinospora cordifolia* extract in human immunodeficiency virus positive patients). Ind J Pharma, 2008; 40(3): 107-110.
42. Thatte UM, Kulkarni MR, Dahanukar SA. (Immunotherapeutic modification of *Escherichia coli* peritonitis and bacteremia by *Tinospora cordifolia*). J Postgrad Med, 1992; 38(1): 13-15.
43. Bapat RD, Rege NN, Koti RS, Desai NK, Dahanukar SA. (Can We Do Away with PTBD?). HPB Surg, 1995; 9(1): 5-11.
44. Roth JA, Kaeberle ML. (Evaluation of bovine polymorphonuclear leukocyte function). Vet Immunol Immunopathol. 1981; 2(2): 157-174.
45. Badar VA, Thawani VR, Wakode PT, Shrivastava MP, Gharpure KJ, Hingorani LL, Khiyani RM. (Efficacy of *Tinospora cordifolia* in allergic rhinitis). J Ethnopharmacol, 2005; 96(3): 445-449.
46. Small P, Kim H. (Allergic rhinitis). Allergy Asthma Clin Immunol. 2011;7(Suppl 1):1-8.
47. Sanis KB, Sumariwalla PF, Goel A, Chintalwar GJ, Sipahimalani AT, Banerji A. Immunomodulatory properties of stem extracts of *Tinospora cordifolia*: Cell targets and active principles. In: Immunomodulation, Upadhyay SN. (ed.). New Delhi: Narosa Publishing House: 1997.
48. Aderem A, Ulevitch RJ. (Toll-like receptors in the induction of the innate immune response). Nature, 2000; 406(6797): 782–787.
49. Brown GD, Gordon S. (Immune recognition. A new receptor for beta-glucans). Nature, 2001; 413(6851): 36–37.
50. Bohn JA, BeMiller JN. ((1→3)-β- d -Glucans as biological response modifiers: a review of structure-functional activity relationships). Carbohydrate Polymers, 1995; 28(1): 3-14.
51. Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction of inflammatory responses by Dectin-1 and Toll-like receptor 2. J Exp Med, 2003; 197(9): 1107–1117.
52. Wang PY, Zhu XL, Lin ZB. (Antitumor and immunomodulatory effects of polysaccharides from broken spore of *Ganoderma lucidum*). Front Pharmacol, 2012; 3(135): 1-8.
53. Nair PKR, Melnick SJ, Ramachandran R& Escalon, Enrique AE, Ramachandran C. (Mechanism of macrophage activation by (1,4)-Alpha-D-glucan isolated from *Tinospora cordifolia*). Int Immunopharmacol, 2007; 6(12): 1815-1824.
54. Aranha I, Clement F, Venkatesh YP. (Immunostimulatory properties of the major protein from the stem of the Ayurvedic medicinal herb, guduchi (*Tinospora cordifolia*)). J Ethno pharmacol, 2011; 139(2). 366-372.
55. Kumar RA, Vaze MB, Chandra NR, Vijayan M, Muniyappa K. (Functional Characterization of the Precursor and Spliced Forms of RecA Protein of *Mycobacterium tuberculosis*). Biochem, 1996: 35(6): 1793-1802.
56. Sinha K, Mishra NP, Singh J, Khanuja SPS. (*Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic applications: A Review). Ind J Trad Knowl, 2004; 3(3): 257-270.
57. Singh N, Singh SM, Shivastava P. (Immunomodulatory effect of *Tinospora cordifolia* in tumor bearing host). Oriental Pharm Experi Med, 2003; 3(2): 72-79.
58. Manjunath N, Shankar P, Wan J, Weninger W, Crowley MA, Hieshima K, Springer TA, Fan X, Shen H, Lieberman J. von Andrian UH. (Effector differentiation is not prerequisite for generation of memory cytotoxic T lymphocytes). J Clin Invest, 2001; 108(6): 871-878.