Ind. J. Pharm. Edu. Res, 2023; 57(2):490-495.
https://www.ijper.org

Original Article

AmruthMune, A Standardized Extract Comprises of Tinosporin from *Tinospora cordifolia* and its Mechanism of Immunostimulatory Activity

Kuluvar Gouthamchandra[1,*], Sudeep Heggar Venkataramana[1], Amritharaj[1], Anusha Sathish[1], Lingaraju Harakanahalli Basavegowda[2], Naveen Puttaswamy[3], Shyam Prasad Kodimule[2,3]

[1]Department of Biomedicinal Research, R&D Centre for Excellence, Vidya Herbs Pvt. Ltd., Bangalore, Karnataka, INDIA.
[2]Department of Phytochemistry, R&D Centre for Excellence, Vidya Herbs Pvt. Ltd., Bangalore, Karnataka, INDIA.
[3]Department of Analytical Development Research Lab, R&D Centre for Excellence, Vidya Herbs Pvt. Ltd., Bangalore, Karnataka, INDIA.

ABSTRACT

Background: *Tinospora cordifolia* is an illustrious medicinal herb in Indian traditional ayurvedic medicine because of its substantial medicinal properties. It is reported that *Tinospora cordifolia* has been used to cure for many ailments by traditional medicinal practitioners. It is captivating that, in this contemporary world *Tinospora cordifolia* is being used to retain good immune health. **Materials and Methods:** The objective of this research is to demonstrate the immune enhancing activity of AmruthMune a standardized extract of *T. cordifolia* extract with Tinosporin. MTT assay, NO assay, H_2O_2 assay, Phagocytosis assay, ELISA, and western blotting experiments were conducted in this study to understand the efficacy and its basic molecular mechanism. **Results:** Quantitative HPLC analysis determined that the AmruthMune a standardized *Tinospora cordifolia* extract comprise of active principle Tinosporin (1%). In addition, AmruthMune treatment dramatically improved the phagocytosis and secretion of H_2O_2 NO, TNF-α, and IL-6 in macrophage cells. Furthermore, consequences of western blot experiments demonstrated that AmruthMune treatment activates p-ERK, p-p38 and p-JNK. **Conclusion:** Collectively, these results exhibited MAPK signalling pathway involved in AmruthMune elicited macrophage activation and it could be a promising functional food in immune modulation.

Keywords: Immune modulation, Murine macrophages, Phagocytosis, ELISA, MAPK signalling pathway.

Correspondence
Dr. K. Gouthamchandra
Department of Biomedicinal Research,
R&D Centre for Excellence,
Vidya Herbs Pvt. Ltd.,
Bangalore-560105, Karnataka, INDIA.
Email id: goutham06@gmail.com
ORCID ID 0000-0002-9029-2187

Received: 28-02-2022;
Revised: 12-07-2022;
Accepted: 03-10-2022.

INTRODUCTION

Body's immune system is comprised of a complicated association of tissues, cells and organs which assist to defend the host from the harmful elements such as parasites, bacteria, fungi, viruses, and cancer cells.[1] Immune system gets triggered, when it finds outside factors or the stimuli which immune system believes the deleterious substance and termed it as antigen. Host generates immune response when it encounters with the antigen through signalling between the two different cells.[2] Immune system is comprised of two types of immune response: innate and adaptive. It is widely understood that the innate immune system made up of functionally diverse mechanism and offers the first line of defence through skin, mucous membranes, or inflammatory components cytokines, and interferons. The adaptive immune response is specific and linked with memory, after exposure to an antigen, examples of cells in the adaptive immune response are B cells and T cells. Immune system plays significant role in keeping the host healthy, when immune system is weak it results in more susceptibility to diseases and is also involved in tumour development.[3-5] Immunomodulators are the substances that may modulate and strengthen the immune system, keeping it particularly prepared for any menace. However, the unpleasant effects of presently used synthetic immunomodulatory agents have stemmed in an increasing significance in the discovery of new, natural compounds without having damaging effects with anti-infectious and immune boosting property.[6] Several medicinal plants have shown an immune stimulatory activity via activating immune cells, encouraging cytokines production, and monitoring the immune system in *in vitro* and *in vivo* models.[7-8] *Tinospora cordifolia* is an herbaceous climber belongs to family Menispermeace and is being used traditionally to combat illness conditions like fever, jaundice, skin infections, diabetes asthma, skin disorders, leprosy, and diarrhoea by traditional medical practitioners. *T. cordifolia* is a rich source secondary metabolites like alkaloids, sesquiterpenoid, glycosides, phenolic

DOI: 10.5530/ijper.57.2.59

Copyright Information
Copyright Author (s) 2023 Distributed under Creative Commons CC-BY 4.0

Publishing partner : EManuscript Tech [www.emanuscript.in]

compounds, steroids, and polysaccharides.[9-11] Ministry of AYUSH, Government of India incorporated *T. cordifolia* in the proposed alternative medicine to improve immunity. It has been reported that raw material samples of *T. cordifolia* available in the market are frequently found to be contaminated with other species of Tinospora like *T. sinensis*. These adulterated samples are missing from the active compounds like Tinosporin and Berberine. It has been reported that Tinosporin a diterpenoid has exhibited antiviral activity against HIV and Herpes simplex Virus (HSV) and other viruses and these viruses steadily diminishes the immune system.[12,13] Therefore, by considering we undertook the present investigation to evaluate the Immunomodulatory activity of AmruthMune a standardized extract containing 1% Tinosporin.

MATERIALS AND MATERIALS

Berberine chloride, Tetrahydropalmatine, Magnoflorine, 20-Hydroxyedysone, Palmatine chloride are used as reference standards. The reagents and solvents used were of analytical and HPLC grade. DMEM medium were bought from Sigma Aldrich. FBS was procured from Gibco BRL (Thermo Fisher Scientific), LPS, antibiotic penicillin–streptomycin solution. All reagents and solvents used were of analytical and HPLC grade. (3-4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Lipopolysaccharide (LPS), antibiotic penicillin–streptomycin solution and Primary antibodies phospho-p38 mitogen-activated protein kinase (MAPK), p38, phosphor-stress-activated protein kinase/Jun amino terminal kinases(p-JNK), JNK, phospho-ERK and ERK).

Standardized extract preparation

The stem part of *Tinospora cordifolia* were collected locally in Bengaluru, India, the samples were air-dried until it achieves steady weight. The five hundred gram of powdered material was then refluxed in 3.5 liters of 50% (v/v) hydro alcohol using three necked round bottom flask for 3-4 hr at temperature of 65-68°C. The mixture was filtered utilizing gauze cloth and the final filtrate was concentrated using a rotary evaporator under reduced pressure into slurry which was dried up additionally in oven to get complete dry extract, the yield of dry extract was 50gm. Further 50gm of dried extract was soaked in 500 ml of 95% alcohol and stirring constantly for 1 hr in room temperature, filtered the extract using filter paper and concentrated the filtrate in a rotary evaporator under vacuum, yield of the dried extract was 5.0 g.

Method of analysis

The quantification of alkaloids was carried out on a Shimadzu LC2030 C Prominence-i (Japan), Kinetex C_{18} column (100 Aº, 250 mm × 4.6 mm, 5 µm pore size) was used for separation. A low-pressure differential is used to elute the mobile phase using 0.1% formic acid in water and acetonitrile, in the ratio (9:10) with time 20 min with flow rate of 1.0 ml/min and 10 µl of injecting volume. The column was kept at 30°C until the completion of analysis, and the wavelength was set to 215 nm. 100% methanol was used as a diluent for HPLC analysis, and overall run time required was 20 min.

Cell culture and treatment

RAW264.7 a murine macrophage cells were purchased from NCI, Pune and were continued to grow using cell culture medium DMEM containing 100 U/ml penicillin and 100µg/ml streptomycin antibiotic solution, 10% fetal bovine serum at 37°C under a humidified atmosphere of 5% CO_2.

Cell viability assay

MTT assay was carried out to assess the effect of AmruthMune on the viability of macrophage RAW264.7 cells by adopting the method.[14] In brief, 2×10^4 cells were set on 96-plate and incubated for 12-14hr. Then, cells were treated with varying concentration of AmruthMune (50-500µg/mL) for 24 hr. After 24 hr, DMEM media containing MTT solution (1 mg/ml) were added to wells and incubated for additional 4-5 hr. After incubation cell culture media was withdrawn, to dissolve formazan crystals, 100µl of DMSO was used. Then, the absorbance was read at 570 nm using Multiscan EX.

Measurement of NO level

Amount of NO secreted from RAW264.7 cells was measured by Griess assay. Briefly, 2×10^4 cells were placed in 96 well plate for 18 hr. Subsequently, cells were exposed with two different concentrations (50 and 100µg/mL) of AmruthMune and incubated for 24 hr. After 24 hr, equal volume of the cell culture supernatants and Griess reagent was mixed and 15-min incubation at room temperature was carried out. After 15 min, the optical density was read at 540 nm using Multiscan EX.

Measurement of H_2O_2 release assay

Assay was carried out by adopting the protocol as described in.[15] In brief, 2×10^4 cells were plated in 96-well plates and exposed with various concentrations of AmruthMune. Assess the H_2O_2 release equal volume of cell culture supernatant of AmruthMune treated cells were mixed with phenol red solution in an equal volume and incubated for 3 hr. finally, the reaction was terminated by simply adding 10µl of 1N NaOH solution. The OD was read at 620nm, the concentration of H_2O_2 determined from the standard curve of H_2O_2 (0-50 µM).

Phagocytosis assay (Neutral Red Uptake Assay)

The ability of phagocytosis was determined by adopting the method neutral red uptake assay.[16] After cells were treated with AmruthMune (at two different concentrations) and LPS (1 µg/mL) for 24 hr, 100µl of 0.075% neutral red solution was added and

kept it in incubator for 1 hr, plates were washed out with PBS twice remove the dye subsequently cell lysis solution comprised of acetic acid and anhydrous alcohol in equal volume was added and the OD was measured at 540nm.

Cytokine assays

For the cytokine assay, RAW264.7 cells were seeded on a 6-well plate at a density of $0.8*10^6$ cells/well and treated with AmruthMune ((50 and 100 µg/mL)) and LPS (1 µg/mL) for 24 hr. As per the manufacturer instructions, TNF-α and IL-6 levels were determined in RAW264.7 cells using ELISA assay kits (Krishgen).

Western blot analysis

The RAW264.7 cells were treated with various concentrations of AmruthMune. The positive control group was LPS only (1µg/mL). Cell lysates were prepared using RIPA buffer. The concentration of protein was determined by Bradford assay (Biorad). Separation of equal amounts of protein loaded on 10% sodium dodecyl sulphate–polyacrylamide gel was performed and then transferred onto PVDF membranes. After blocking with skimmed milk, the membrane was probed with the primary antibody against p-p38, p-JNK and p-ERK obtained from Santacruz, and horseradish peroxidase-conjugated secondary antibodies. Signals were caught with the enhanced chemiluminescence (ECL) kit.

Statistical analysis

The data of the experiments are expressed as a mean ± standard deviation. Using GraphPad Prism 9.0, we carried out an ANOVA and Tukey's Multiple-Comparison test to ascertain the statistical significance. Significant differences were examined at $p < 0.05$.

RESULTS

HPLC analysis

We were able to confirm the peak identification for Tinosporin, Berberine chloride, Tetrahydropalmatine, Magnoflorine, 20-Hydroxyedysone, Palmatine chloride standards and samples to be at 8.156, 6.508, 6.035, 5.124, 5.690, 6.410 and 8.183, 6.50. shown in Figure 1. The achieved percentage assay of each individual alkaloids in *Tinospora cordifolia* extract is as shown in Table 1.

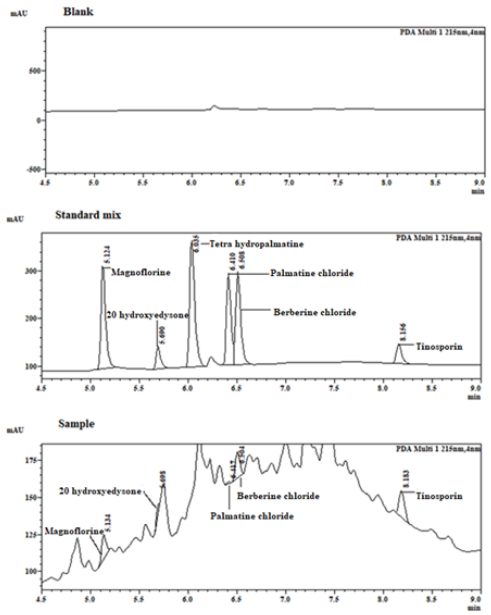

Figure 1: Optimised HPLC Chromatograms of blank, standard, and sample.

Table 1: Percentage of alkaloids content in *Tinospora cordifolia* sample.

Name of the compound	Assay reached (%)
Tinosporin	1.22
Berberine chloride	1.16
Tetrahydropalmatine	Not detected
Magnoflorine	0.81
20-Hydroxyedysone	0.41
Palmatine chloride	0.12

Effect of AmruthMune on the cell viability and Nitric Oxide production

Initially, we attempted to ascertain the non-cytotoxic concentration of the AmruthMune a standardized *T. cordifolia* extract comprising of 1% tinosporin. Find out whether AmruthMune affects cell viability, we conducted the MTT assay. We observed that AmruthMune did not affect cellular proliferation up to the concentration of 400µg/mL whereas at higher concertation AmruthMune showed moderate toxicity (Figure 2A). Nitric oxide (NO) plays a key role in immune responses as a physiological messenger. Study the outcome of AmruthMune on production of NO in macrophages, we determined the nitrite (NO_2) levels in cell culture supernatant. Interestingly we observed significant enhancement in the production of NO at the

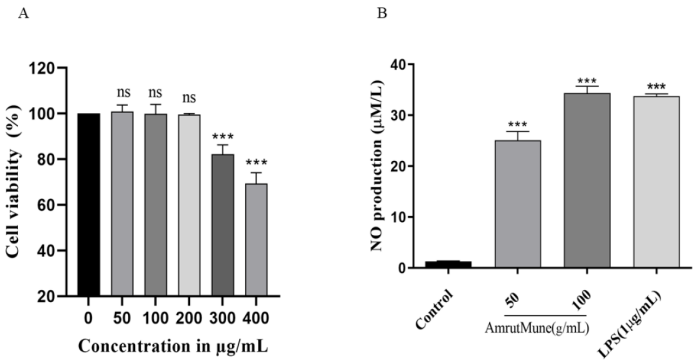

Figure 2: Effect of AmruthMune on cell viability and production of nitric oxide (NO). Various concentration of AmruthMune were incubated with RAW 264.7 cells for 24 hr, cell viability was measured based on MTT assay (A). Griess reaction was used to determine NO levels in the culture media (B). Data represent means ± standard error of the mean of three independent experiments. ***$p < 0.0001$, significant as compared to the control group.

concentration of 100µg/mL of AmruthMune (Figure 2B) which is equivalent to LPS (positive control).

Influence of AmruthMune on the H_2O_2 production of macrophages

We have noticed the release of H_2O_2 found to be concentration dependent, at the concentration of 50 and 100 µg/mL the release of H_2O_2 was 21.66 and 29.74 µM respectively (Figure 3A). While LPS (1µg/mL) treated cells showed 29.74 µM (positive control).

Influence of AmruthMune on the phagocytosis of RAW264.7 cells

It is demonstrated that macrophages are key phagocytes, macrophages offer defence against pathogens entered through phagocytosis. Influence of AmruthMune on the phagocytosis of murine macrophages RAW264.7 cells were exhibited in Figure 3B. Optical density of AmruthMune extract treated macrophage cells significantly higher than the normal control group. AmruthMune treatment at the concentration of 100µg/mL would be able to enhance the level of phagocytosis which is almost equivalent to LPS treated RAW 264.7 macrophages.

Impact of AmruthMune on the macrophage's secretion and expression of cytokines

TNF-α and IL-6 are the major proinflammatory cytokines secreted by macrophages. Hence, the influence of AmruthMune on the cytokines production in RAW 264.7 cells were determined using the cell culture media by ELISA. As presented in Figure 4, the secretion of cytokines TNF-α and IL-6 were enhanced considerably in the presence of AmruthMune. At the concentration of 100µg/mL, TNF-α (Figure 4A) and IL-6 (Figure 4B), in the cell culture media of treated RAW 264.7 cells were higher than LPS-treated cells. These data indicate that the

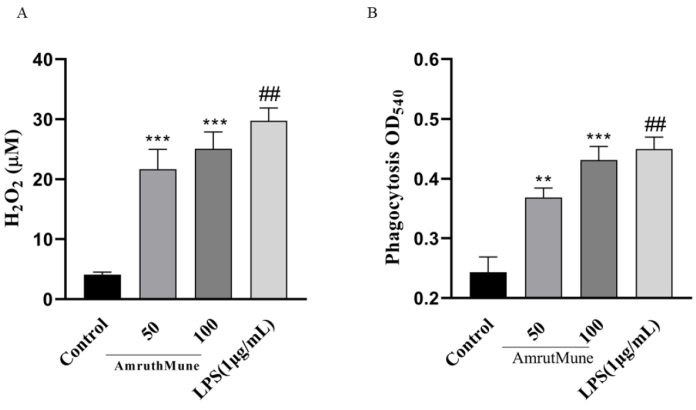

Figure 3: An evaluation of the effects of AmruthMune on H_2O_2 release and phagocytosis of RAW264.7 cells. Data presented as "mean ± standard error" from three independent experiments. ***$p < 0.0001$, significant as compared to the control group. ##p <0.0001 < significant compared to control group.

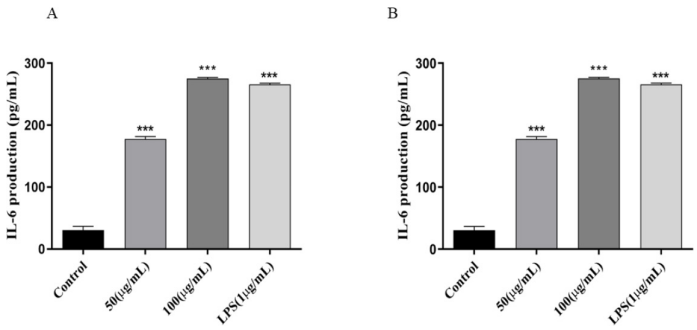

Figure 4: AmruthMune induced TNF-α and IL-6 production in murine macrophages RAW264.7 cells treated with AmruthMune (50 and 100 µg/ml). Data represent the mean ± standard error of three experiments. ***$p < 0.0001$, significant as compared to the control group. ##$p < 0.0001$ < significant compared to control group.

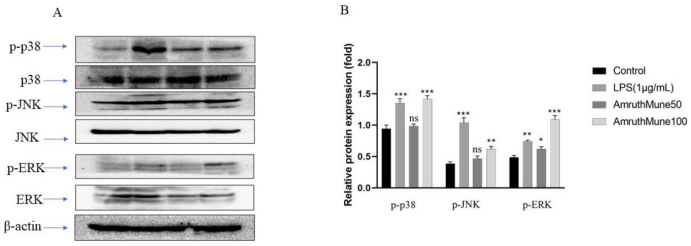

Figure 5: Effects of AmruthMune on MAPKs signalling pathway in RAW 264.7 cell. The protein expression levels of ERK1/2, p-ERK1/2, JNK, p-JNK, p38 and p-p38 were identified based on their antibodies. Data were expressed as mean ± SD ($n = 3$). Significant difference from the control group was designated as *$p < 0.05$, **$p < 0.001$, **$p < 0.0001$.

cytokine secreting effect of AmruthMune are found to be effective compared to untreated RAW 264.7 cells.

Effect of AmruthMune on MAPKs signaling pathways of RAW 264.7 macrophages

We have assessed the effect of AmruthMune on MAPK signalling pathway using western blot experiments. As exhibited in Figure 5 AmruthMune treatment elicits the phosphorylation of all the three, ERK, JNK and p38 (MAPKs). It is noticeably clear that AmruthMune at the concentration of 100µg/mL phosphorylates ERK, JNK and p38, whereas no changes observed in the total basal form of ERK, JNK and p38. It is noticeably clear from the study that AmruthMune activates MAPKs, and the levels of MAPKs were almost equivalent to LPS caused phosphorylation.

DISCUSSION

The whole plant *T. cordifolia* is reported to possess antioxidant, antiarthritic, antidiabetic, anti-inflammatory, antiulcer, weakness, dyspepsia, fever, inflammation, jaundice, urinary, skin. Fascinatingly that the annual consumption of *T. cordifolia* in the Indian System of Medicines is 1,000 tonnes.[17-19] Initially we conducted simple extraction procedure to confirm the presence of berberine a biomarker compound of *T. cordifolia*. Further, we have determined the cytotoxicity of AmruthMune a standardized

extract of *T. cordifolia* on the RAW264.7 macrophages, the results ascertained that AmruthMune did not show toxic effects on macrophages. Based on this, successive studies were conducted by choosing the safe medication dose of AmruthMune i.e., 50 and 100µg/mL.

NO was found to be a critical component of the immune system. There is evidence to support the secretion of NO by macrophages exhibited antipathogen and antitumor activity.[20-21] Therefore, NO measurement can reflect the effects of AmruthMune on immune stimuli, which could be used as a quantitative marker of macrophage activation. Previous studies have reported that the various types of extracts of *T. cordofolia* are proved to enhance the phagocytic activity of macrophages and it also increases the activation rate of macrophages.[22-23] The enhancement of phagocytosis is the distinctive feature of macrophage activation, the stimulation of macrophages enables the production of a variety of immunomodulatory substances involving cytokines, such as TNF-α and IL-6 triggering an array of innate immune responses to invading pathogens.[24]

In this study, AmruthMune demonstrated macrophages stimulation and the effect was comparable with LPS, AmruthMune enhanced the production of IL-1β and IL-6 in murine macrophages at the concentration of 100µg/mL. These findings suggest that AmruthMune had a strong immunomodulation effect on macrophages *in vitro*. Our results were coherent with the earlier published studies showing macrophage activation by LPS.[25] Understand the mechanism behind the activation of pro-inflammatory cytokines and increased production of NO. We evaluated effect of AmruthMune on activation of MAPK signalling pathway. It has been reported that mitogen activated protein kinase (MAPK) plays a key role in innate and adoptive immune response.[26-27] Studies in connection with the immunomodulating materials reported they trigger macrophages via MAPK pathways.[28-29] In our investigation we also found that the phosphorylation of ERK, JNK and p38 upon treatment with AmruthMune a standardized extract of *Tinospora cordifolia* which is almost equal to LPS, the above results revealed the molecular mechanisms of AmruthMune in the activation of MAPK pathways in murine macrophages. Results were in line with previously published studies.[30-31]

CONCLUSION

In conclusion, AmruthMune helped the maturation of macrophages without toxicity, encouraged NO and H_2O_2 production in RAW 264.7 macrophages and elicited the cytokines TNF-α and IL-6 related to pro-inflammation. Interestingly we also observed that all three MAPKs pathways have been linked with macrophage activation. The above results suggest that AmruthMune is potential immune-stimulating standardized extract. Therefore, AmruthMune can be used in the food or pharmaceutical industry. However, more detailed *in vivo* study is necessary to ensure its benefits.

ACKNOWLEDGEMENT

We would like to thank, Mr. Chandrappa S, Dr. Deepak, and Dr. Vedamuthy B.M, Department of Phytochemistry and Analytical Development Laboratory, Vidya Herbs Pvt. Ltd., Bangalore, India. Our lab members are also acknowledged for their useful suggestions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FBS: Fetal bovine serum; **LPS:** Lipopolysaccharide; **MTT:** (3-4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **MAPK:** Mitogen-activated protein kinase: **NO:** Nitric oxide; **H_2O_2:** Hydrogen peroxide.

SUMMARY

In Indian ayurvedic medicine, *Tinospora cordifolia* is regarded highly as a therapeutic herb due to its insightful therapeutic properties. In this study we have demonstrated the immune enhancing activity of AmruthMune a standardized extract of *T. cordifolia* extract with Tinosporin by conducting the appropriate experiments to understand the efficacy and its basic molecular mechanism. AmruthMune a standardized extract of *T. cordifolia* extract showed immune stimulatory activity by stimulating macrophage activation and it could be a promising functional food in immune modulation.

REFERENCES

1. Calder PC. Feeding the immune system. Proc Nutr Soc. 2013;72(3):299-309. doi: 10.1017/S0029665113001286, PMID 23688939.
2. Grigore A. Plant phenolic compounds as immunomodulatory agents. In: Phenolic compounds—biological activity. London, UK: IntechOpen; 2017.
3. Janeway CA Jr, Travers P, Walport M, *et al*. Immunobiology: The immune system in health and disease. 5th ed. New York: Garland Publishing Science; 2001. Principles of innate and adaptive immunity.
4. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007;449(7164):819-26. doi: 10.1038/nature06246, PMID 17943118.
5. Krystel WM, Dileepan KN. Mast cell WJG. A multi-functional master cell. Front Immunol. 2016;6:620.
6. Patil, U.S. and Jaydeokar, A.V. and Bandawane, D.D. Immunomodulators: A pharmacological review. Int J Pharm Pharm Sci. 2012;4:30-6.
7. Mileo AM, Nisticò P, Miccadei S. Polyphenols: Immunomodulatory and therapeutic implication in colorectal cancer. Front Immunol. 2019;10:729. doi: 10.3389/fimmu.2019.00729, PMID 31031748.
8. Chang Y, Guo A, Jing Y, Lin J, Sun Y, Kong L, *et al*. Immunomodulatory activity of puerarin in RAW264.7 macrophages and cyclophosphamide-induced immunosuppression mice. Immunopharmacol Immunotoxicol. 2021;43(2):223-9. doi: 10.1080/08923973.2021.1885043, PMID 33583301.
9. Yates CR, Bruno EJ, Yates MED. Tinospora cordifolia: A review of its immunomodulatory properties. J Diet Suppl. 2022;19(2):271-85. doi: 10.1080/19390211.2021.1873214, PMID 33480818.
10. Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon. 2019 Sep 12;5(9):e02437. doi: 10.1016/j.heliyon.2019.e02437, PMID 31701036.
11. Singh D, Chaudhuri PK. Chemistry and pharmacology of *Tinospora cordifolia*. Nat Prod Commun. 2017;12(2):299-308. doi: 10.1177/1934578X1701200240, PMID

30428235.

12. Chetan B, Nakum A; 2010. Use of natural compounds, chitin and tinosporin for the treatment of the targeted viruses (retroviruses) (HIV-1, HIV-2) all subgroups, HTLV and other viral disease. Indian patent Appl IN. 2010MU01350. p. A20100730.
13. Singh B, Nathawat S, Sharma RA. Ethnopharmacological and phytochemical attributes of Indian Tinospora species: A comprehensive review. Arab J Chem. 2021;14(10). doi: 10.1016/j.arabjc.2021.103381.
14. Lee JH, Moon SH, Kim HS, Park E, Ahn DU, Paik HD. Antioxidant and anticancer effects of functional peptides from ovotransferrin hydrolysates. J Sci Food Agric. 2017;97(14):4857-64. doi: 10.1002/jsfa.8356, PMID 28382654.
15. Toffoli-Kadri MC, Carollo CA, Lourenço LD, Felipe JL, Néspoli JH, Wollf LG, *et al*. *In vivo* and *in vitro* anti-inflammatory properties of *Achyrocline alata* (Kunth) DC. J Ethnopharmacol. 2014;153(2):461-8. doi: 10.1016/j.jep.2014.03.008, PMID 24632016.
16. Chen W, Zhang W, Shen W, Wang K. Effects of the acid polysaccharide fraction isolated from a cultivated *Cordyceps sinensis* on macrophages *in vitro*. Cell Immunol. 2010;262(1):69-74. doi: 10.1016/j.cellimm.2010.01.001, PMID 20138259.
17. Saha S, Ghosh S. *Tinospora cordifolia*: One plant, many roles. Anc Sci Life. 2012;31(4):151-9. doi: 10.4103/0257-7941.107344, PMID 23661861.
18. Tiwari P, Nayak P, Prusty SK, Sahu PK. Phytochemistry and pharmacology of *Tinospora cordifolia*: A review. Syst Rev Pharm. 2018;9(1):70-8. doi: 10.5530/srp.2018.1.14.
19. Tanwar, S. and Jain, Jainendra and Verma, S. and Solanki, D. Standardization and phytochemical evaluation of *Tinospora cordifolia* (Willd.) miers. (Menispermaceae). Int J Pharm Pharm Sci. 2012;4:219-23.
20. Hibbs JB, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: A cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun. 1988;157(1):87-94. doi: 10.1016/s0006-291x(88)80015-9, PMID 3196352.
21. Gordon S. Phagocytosis: An immunobiologic process. Immunity. 2016 Mar 15;44(3):463-75. doi: 10.1016/j.immuni.2016.02.026, PMID 26982354.
22. Ranjith MS, Ranjitsingh AJ, Gokul Shankar S, Vijayalaksmi GS, Deepa K, Sidhu HS. Enhanced phagocytosis and antibody production by *Tinospora cordifolia*: A new dimension in immunomodulation. Afr J Biotechnol. 2008;7:81-5.
23. Gupta RS, Sharma A. Antifertility effect of *Tinospora cordifolia* (Willd.) stem extract in male rats. Indian J Exp Biol. 2003;41(8):885-9. PMID 15248490.
24. Arango Duque G, Descoteaux A. Macrophage cytokines: Involvement in immunity and infectious diseases. Front Immunol. 2014;5(5):491. doi: 10.3389/fimmu.2014.00491, PMID 25339958.
25. Ilyas U, Katare DP, Naseef PP, Kuruniyan MS, Elayadeth-Meethal M, Aeri V. Immunomodulatory Activity of *Phyllanthus maderaspatensis* in LPS-Stimulated Mouse Macrophage RAW 264.7 cells. Separations. 2021;8(9):129. doi: 10.3390/separations8090129.
26. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. Annu Rev Immunol. 2002;20:55-72. doi: 10.1146/annurev.immunol.20.091301.131133, PMID 11861597.
27. Rincón M, Flavell RA, Davis RA. The JNK and P38 MAP kinase signaling pathways in T cell-mediated immune responses. Free Radic Biol Med. 2000;28(9):1328-37. doi: 10.1016/s0891-5849(00)00219-7, PMID 10924852.
28. Jeong JH, Jang S, Jung BJ, Jang KS, Kim BG, Chung DK, *et al*. Differential immune-stimulatory effects of LTAs from different lactic acid bacteria via MAPK signaling pathway in RAW 264.7 cells. Immunobiology. 2015;220(4):460-6. doi: 10.1016/j.imbio.2014.11.002, PMID 25433634.
29. Hong SH, Ku JM, In Kim H, Ahn CW, Park SH, Seo HS, *et al*. The immune-enhancing activity of *Cervus nippon* mantchuricus extract (NGE) in RAW264.7 macrophage cells and immunosuppressed mice. Food Res Int. 2017;99(1):623-9. doi: 10.1016/j.foodres.2017.06.053, PMID 28784525.
30. Kim GT, Tran NK, Choi EH, Song YJ, Song JH, Shim SM, *et al*. Immunomodulatory efficacy of standardized *Annona muricata* (Graviola) Leaf extract via activation of mitogen-activated protein kinase pathways in RAW 264.7 macrophages. Evid Based Complement Alternat Med. 2016;2016:2905127. doi: 10.1155/2016/2905127, PMID 28096884.
31. Jung HG, Kim HH, Dey DK, Yu JM, Kim H, Cho Y, *et al*. The anti-inflammatory and immune-boosting potential of Quercetin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside in LPS–stimulated RAW264.7 macrophages. Rev Bras Farmacogn. 2020;30(2):233-9. doi: 10.1007/s43450-020-00027-3.

Cite this article: Gouthamchandra K, Venkataramana SH, Amritharaj, Sathish A, Basavegowda LH, Puttaswamy N, Kodimule SP. AmruthMune, A Standardized Extract Comprises of Tinosporin from *Tinospora cordifolia* and its Mechanism of Immunostimulatory Activity. Indian J of Pharmaceutical Education and Research. 2023;57(2):490-5.