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Research Article

IN VITRO IMMUNOSTIMULATION ACTIVITY OF *NIGELLA SATIVA* LINN. AND *PSORALEA CORYLIFOLIA* LINN. SEEDS USING A MURINE MACROPHAGE CELL LINE

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ABSTRACT

Objective: The aim of this study is to analyze the phytochemical constituents and the *in vitro* immunomodulatory potential of ethanol extract of *Nigella sativa* and *Psoralea corylifolia* seeds.

Methods: Phytoconstituents in the ethanol extract of *N. sativa* and *P. corylifolia* seeds were analyzed using gas chromatography and mass spectrum and their immunomodulatory activity was investigated *in vitro* by cell proliferation assay with J774A.1 cell line.

Results: Thirteen biochemical constituents have been identified with ethanol extract of *N. sativa* and nine from the *P. corylifolia*. The maximum relative activity of 138.77±0.44% cell proliferation was expressed at 25 µg/ml by *N. sativa* and 80.70±0.42% of proliferation by *P. corylifolia* at 25 µg/ml.

Conclusion: The results suggest that the ethanol extract of the plant seeds have stimulating activity on macrophage cells and could be useful for modulating immune functions.

Keywords: Immunomodulatory, Cell proliferation, Macrophage cell line, *Nigella sativa*, *Psoralea corylifolia.*

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INTRODUCTION

The immune system is a remarkably versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and to eliminate diseases. The function and the efficacy of immune system may be influenced by many exogenous factors such as food and pharmaceuticals, physical and psychological stress and hormones. Resulting in either immunostimulation or immunosuppression [1]. Suppression in immune response disrupts host's ability to defend itself against bacteria, viruses, and parasites. Immunomodulation using plant material can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism has to be activated under the condition of impaired immune response [2]. A large number of drugs in use are derived from plants, like morphine from *Papaver somniferum*, Aswagandha from *Withania somnifera*, Ephedrine from *Ephedra vulgaris*, Atrophine from *Atropa belladonna,* etc. [3]. The phytochemical constituents like terpenoids, steroids, proteins, and tannins [4] are considered to exhibit this immunomodulatory property.

A large number of plants with therapeutic potentials have been considered. One such is *Nigella sativa* Linn. (Ranunculaceae), commonly known as "black cumin," is an herbaceous plant that grows in Mediterranean countries. The oil and seed constituents have shown potential medicinal properties in traditional medicine [5]. The black seeds contain 36-38% fixed oil, with proteins, alkaloids, saponins, and essential oils making up the rest of the composition [6]. Black seed extract has been reported to possess antimicrobial activity [7], antitumor activity [8], and a stimulatory effect on the immune system [9]. *Psoralea corylifolia* Linn. commonly known as "*Bakuchi*" is conventionally used in ayurvedic system of medicine for the treatment of various kinds of human disorders but especially for treatment of skin disorders [10]. *P. corylifolia* seed has been reported to contain several phytoconstituents and possess antibacterial, antifungal, antioxidant, antiflarial, estrogenic, and immunomodulatory activity [11]. However, there is no scientific data on the *in vitro* immunomodulatory activity of these plants. Therefore the aim of the present study is to identify the bioactive components of these plants by subjecting the ethanol extracts of the plant seeds to gas chromatography and mass spectrum (GC-MS) analysis and to investigate their immune stimulation potential using an *in vitro* cell line model.

METHODS

Collection of plant materials

Seeds of *N. sativa* and *P. corylifolia* were procured from herbal medicine store in Chennai. The seeds were authenticated at the Department of Medicinal Botany, National Institute of Siddha, Chennai and a voucher specimen (voucher number - NISMB2052015) was submitted.

Preparation of plant extracts

Fine seeds were cleaned and milled to a powder using a domestic blender. A total of 25 g of powdered seeds were then subjected to soxhlet extraction with 250 ml of ethanol as solvent. The seed extract was concentrated using vacuum rotatory evaporation to void of solvent. The extracts were filter sterilized and stored at 4°C until further use [12,13].

Cell culture

J774A.1, a murine macrophage cell line, was procured from National Centre for Cell Science, Pune, Maharashtra, India. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) inactivated fetal bovine serum (FBS), 100 units/ml of penicillin, 100 µg/ml of streptomycin and 3% L-glutamine (Himedia Labs, Mumbai). The cells were incubated at 37°C in a humidified atmosphere containing 5% (v/v) carbon dioxide (CO_2). The medium was changed every 2 days, and the cells were serially passaged biweekly [14].

Treatment of cell cultures with ethanol extracts

A volume of 100 µl of medium containing 1×10^5 cells were seeded triplicates in 96-well plates. The plates were incubated for 24 hrs at

37°C in 5% CO_2. After incubation, the old medium was decanted and all the plates were replenished with fresh DMEM medium supplemented with 10% FBS. The cells were then treated with varying concentrations (6.25, 12.5, 25, 50, and 100 µg/ml) prepared by diluting the extract in DMEM and then incubated. The effect of ethanol extracts on macrophage proliferation was evaluated with wells containing only cells as a negative control and compared to cells treated with concanavalin-A (Con-A), positive control [15].

Cell proliferation assay using (3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide) (MTT) reagent

After incubation, 20 µl of MTT reagent (5 mg/ml in phosphate buffer saline) was added to each well and further incubated for 4 hrs at 37°C. Then, dimethyl sulfoxide was added to dissolve the formazon crystals, intensity of the colored reaction product was measured at 540 nm using Microplate Reader (BIOTEK, USA) [16].

GC-MS analysis

GC-MS analysis of the ethanol extract of *N. sativa and P. corylifolia* was carried out with 2 µl of extract employed on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1 EM df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minutes, to 200°C/minutes, then 5°C/minutes to 280°C/minutes, ending with a 9 minutes isothermal at 280°C. MS were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. The MS of the unknown component was compared with the spectrum of the known components stored in The National Institute of Standards and Technology (NIST) library [17,18]. The name, molecular weight, and structure of the components of the test materials were ascertained.

Statistical analysis

The data were analyzed using one-way analysis of variance (one-way ANOVA). Results expressed as mean±standard deviation for triplicate assays using Graph Pad Prism software. Values of $p<0.05$ were the criteria for statistical significance.

RESULTS

The GC-MS analysis of the ethanol extract of *N. sativa* seeds revealed the presence of 13 peaks (Fig. 1) and *P. corylifolia* showed 9 peaks (Fig. 2) indicating the presence of phytoconstituents whose name, molecular weight and structure were identified and characterized on comparison with data in the NIST library (Tables 1 and 2).

Cell proliferation assay

Ethanol extract of *N. sativa* showed a maximum relative activity of 138.77±0.44% at 25 µg/ml compared to the standard drug (Con-A) which expressed a proliferation rate of 51.11±0.96%. 11.30±0.24, 112.03±0.15, 138.77±0.44, 83.46±0.30 and 50.54±0.43% increase in the proliferation of macrophage cells were reported at the dose rate of 6.25, 12.5, 25, 50, and 100 µg/ml of ethanol extract, respectively (Fig. 3). The ethanol extract of *P. corylifolia* showed a maximum relative activity of 80.70±0.42% at 25 µg/ml compared to the standard (Con-A), resulting in the proliferation range of 10.17±0.76, 28.46±0.46, 80.70±0.42, 38.02±0.55, and 12.16±0.23% between varying concentrations of 6.25, 12.5, 25, 50, and 100 µg/ml (Fig. 4).

DISCUSSION

The information regarding the chemical constituents of a plant is generally provided by the qualitative phytochemical screening of its extract. Hence, the GC-MS analysis was opted to assess the phytochemical constituents present in the ethanol extracts of the seeds. Among the 13 components characterized from the GC-MS analysis of *N. sativa.* 9,12-octadecadienoic, ethyl ester was identified as a major chemical constituent (50.98%) followed by Hexadecanoic acid, ethyl ester (19.63%) and 8-octadecenoic acid, methyl ester,(E)- (14.56%) in the ethanol extract of *N. sativa*. Phenol, 4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)- (45.99%) and Linoleic acid ethyl ester (45.99%) was identified as the major chemical constituent in the ethanol extract of *P. corylifolia.* Other components in trace amounts were also present in the ethanol extract of both plant seeds analyzed.

The immunomodulatory effect in the cell proliferation model has been a target of study in the search for new therapeutic agents of natural origin [19,20]. Although various components have been screened for their immunomodulatory potential using cell line as a model, in this study, immune stimulating activity of ethanol extract of *N. sativa and*

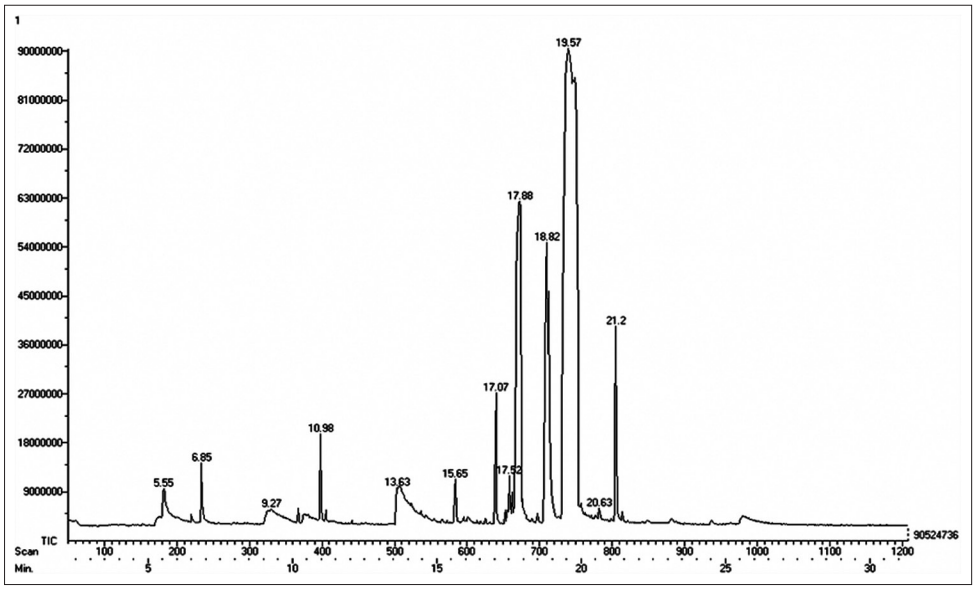

Fig. 1: Chromatogram of ethanol extract of *Nigella sativa* seed by gas chromatography and mass spectrum

Table 1: Chemical composition (%) of ethanol extract of *Nigella sativa* seed

S. No.	Name of the compound	Retention time	Peak %	Molecular weight g/mol	Chemical formula
1	o-cymol	5.5	1.66	134.21	$C_{10}H_{14}$
2	Ether, p-menth-6-en-2-yl methyl	6.85	1.13	168.27	$C_{11}H_{20}O$
3	(+)-Longifolene	10.98	0.99	204.35	$C_{15}H_{24}$
4	Pentadecanoic acid, 14-methyl-, methyl ester	17.08	2.34	270.45	$C_{17}H_{34}O_2$
5	8-Octadecenoic acid, methyl ester,(E)-	18.82	14.56	296.48	$C_{19}H_{36}O_2$
6	9,12-Octadecadienoic, ethyl ester	19.57	50.98	308.49	$C_{20}H_{36}O_2$
7	10,13-Eicosadienoic acid, methyl ester	20.63	0.36	322.52	$C_{21}H_{38}O_2$
8	1-Tetradecene, 2-decyl-	21.23	3.42	336.63	$C_{24}H_{48}$
9	2,5-Cyclohexadiene-1,4-dione, 2-methyl-5-(1-methylethyl)-	9.27	1.03	164.20	$C_{10}H_{12}O_2$
10	Durohydroquinone	13.7	2.40	166.22	$C_{10}H_{14}O_2$
11	Ethyl 9-hexadecenoate	17.52	0.90	282.46	$C_{18}H_{34}O_2$
12	Hexadecanoic acid, ethyl ester	17.93	19.63	284.47	$C_{18}H_{36}O_2$
13	Tetradecanoic acid, ethyl ester	15.65	0.83	256.42	$C_{16}H_{32}O_2$

Table 2: Chemical composition (%) of ethanol extract of *Psoralea corylifolia* seed

S. No.	Name of the compound	Retention time	Peak %	Molecular weight g/mol	Chemical formula
1	Caryophyllene	11.50	1.81	204.35	$C_{15}H_{24}$
2	Epoxycaryophyllene	13.32	1.92	220.35	$C_{15}H_{24}O$
3	Estra-1,3,5 (10)-trien-17a-ol	17.77	1.13	286.40	$C_{19}H_{26}O_2$
4	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	18.75	1.91	294.47	$C_{19}H_{34}O_2$
5	Phenol,4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)-	19.30	45.99	256.38	$C_{18}H_{24}O$
6	Linoleic acid ethyl ester	19.45	45.99	308.49	$C_{20}H_{36}O_2$
7	11-Eicosenoic acid, methyl ester	20.60	0.85	324.54	$C_{21}H_{40}O_2$
8	13-Docosenoic acid, methyl ester, (Z)-	22.53	0.39	352.59	$C_{23}H_{44}O_2$
9	Ethyl 13-docosenoate (ethyl erucate)	23.28	0.34	366.62	$C_{24}H_{46}O_2$

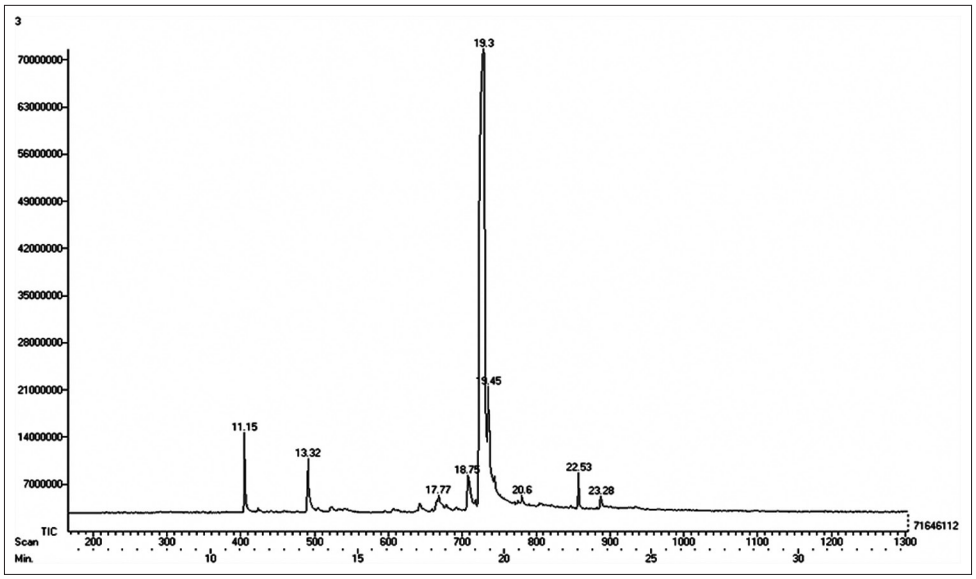

Fig. 2: Chromatogram of ethanol extract of *Psoralea corylifolia* seed by gas chromatography and mass spectrum

P. corylifolia have been screened using J774A.1 cell line. Ethanol extract of *N. sativa* and *P. corylifolia* seeds assessed for cell proliferation assay, induced an increase in cell proliferation depending on the varying concentrations. In previous reports, 6-carboxymethylthiopurine, an anti-inflammatory drug and the hot-water extract of *Sargassum hemiphyllum* was reported to stimulate proliferation of J774A.1 cell [21,22]. The proliferation stimuli were dose dependent in earlier reports, where maximum proliferation was obtained from higher concentration. The reports were in contrast to the current study where the extract at its maximum and minimum concentration had a varying effect on the macrophage cells. Percentage of proliferation was comparatively lower at both concentration. The present observation might be due to toxic nature of the analyzed samples at higher concentration while the minimum concentration would have had a negligible effect on cell duplication.

Present results indicate that the tested ethanol extract of *N. sativa* and *P. corylifolia* are capable of stimulating the immune function by increasing the proliferation rate of macrophages. Purification and isolation of the component responsible for activity were required to void the toxic component in the crude extract to construct potential immunomodulator agent.

CONCLUSION

The phytochemical analyze concludes that *N. sativa and P. corylifolia* seed extract contain various bioactive compounds. The study is evident

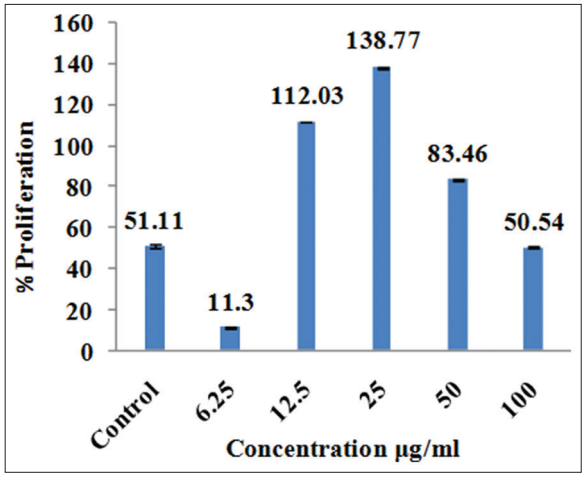

Fig. 3: The stimulating effect of the ethanol extract of *Nigella sativa* on J774A.1 cells. The data plotted represent mean±standard deviation of triplicate experiments (n−3). p<0.05

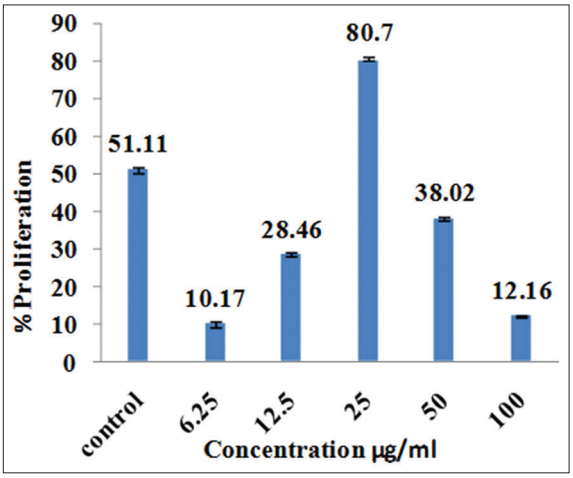

Fig. 4: The stimulating effect of the ethanol extract of *Psoralea corylifolia* on J774A.1 cells. The data plotted represent mean±standard deviation of triplicate experiments (n−3). p<0.05

that ethanol extract of *N. sativa* and *P. corylifolia* played an important role in the modulation of the immune response and thus may have application as an immunomodulatory agent.

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REFERENCES

1. Patil VV, Bhangale CS, Patil RV. Studies on immunomodulatory activity of *Ficus carica*. Int J Pharm Pharm Sci 2010;2(4):97-9.
2. Chitra V, Janaki PS, Raju D, Rao PV. Evaluation of immunomodulatory activity of ethanolic extract of leaves of *Glycosmis pentaphylla* in Swiss albino mice. Int J Pharm Pharm Sci 2013;5(4):110-3.
3. Bhadoriya SS, Mangal A, Mandoriya N. Immunomodulatory effect of *M. tricuspidatum*. Int J Curr Pharm Res 2012;4(2):33-6.
4. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Curr Sci 2002;82(11):1136-345.
5. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int Immunopharmacol 2005;5(13-14):1749-70.
6. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 2000;14(5):323-8.
7. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiol Pol 2000;49(1):63-74.
8. Worthen DR, Ghosheh OA, Crooks PA. The *in vitro* anti-tumor activity of some crude and purified components of blackseed, *Nigella sativa* L. Anticancer Res 1998;18(3A):1527-32.
9. Salem ML, Hossain MS. *In vivo* acute depletion of CD 8 (+) T cells before murine cytomegalovirus infection upregulated innate antiviral activity of natural killer cells. Int J Immunopharmacol 2000;22(9):707-18.
10. Siddhinandan M. Khadirarishta-Kushtharogadhikar. 54th ed. Varanasi: Bhiashajya Ratnavali Chaukhambha Publication; 2009. p. 353-8.
11. Latha PG, Panikkar KR. Inhibition of chemical carcinogenesis by *Psoralea corylifolia* seeds. J Ethnopharmacol 1999;68(1-3):295-8.
12. Chanda S, Kaneria M, Nair R. Antibacterial activity of *Psoralea corylifolia* Linn. Seed and aerial parts with various extraction methods. Res J Microbiol 2011;6(2):124-31.
13. Ozcan M. Inhibitory effects of spice extracts on the growth of *Aspergillus parasiticus* NRRL2999 strain. Z Lebensm Unters Forsch A 1998;2079(3):253-5.
14. Musso T, Badolato R, Ravarino D, Stornello S, Panzanelli O, Merlino C, *et al*. Interaction of *Bartonella henselae* with the murine macrophage cell line J774: Infection and proinflammatory response. Infect Immun 2001;69(10):5974-80.
15. Goel A. Immumological Studies on Fileriaris, Ph.D. Thesis. Lucknow, Uttar Pradesh, India: Central Drug Research Institute, (CDRI); 1991.
16. Snedecor GW, Cochran WG. Statistical Methods. 7th ed. Ames, Iowa, USA: The Iowa State University; 1981.
17. Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. Chemical investigation of aerial parts of *Gmelina asiatica* Linn. by GC-MS. Pharmacogn Res 2009;1(3):152-6.
18. Anand D, Wyson WJ, Saravanan P, Rajarajan S. Phytochemical analysis of leaf extract of *Eclipta alba* (L.) Hassk by GC-MS method. IJPPR 2014;6(3):562-6.
19. Arokiyaraj S, Perinbam K, Agastian P, Balaraju K. Immunosuppressive effect of medicinal plants of Kolli Hills on mitogen-stimulated proliferation of the human peripheral blood mononuclear cells *in vitro*. Indian J Pharmacol 2007;39(4):180-3.
20. Sriwanthana B, Treesangsri W, Boriboontrakul B, Niumsakul S, Chavalittumrong P. *In vitro* effects of Thai medicinal plants on human lymphocyte activity. Songklanakarin J Sci Technol 2007;29(1):17-28.
21. De Paula ML, Braga FG, Coimbra ES, Carmo AM, Teixeira HC, Da Silva AD, *et al.* Modulatory effects of 6-carboxymethylthiopurine on activated murine macrophages. Chem Biol Drug Des 2008;71(6):563-7.
22. Hwang PA, Chwen-Herng WU, Gau SY, Chien SY, Hwang DF. Antioxidant and immune-stimulating activities of hot-water extract from Seaweed *Sargassum hemiphyllum*. JMST 2010;18(1):41-6.