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IDENTIFICATION OF PLANT SOURCES FROM NORTH ANDHRA PRADESH EXHIBITING IMMUNOMODULATORY ACTIVITY USING BALB/c MODELS

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ABSTRACT

In this present study, 10 different medicinal plants from north Andhra Pradesh were selected for the identification of potential immunomodulatory property using Balb/c mice. The aqueous leaf extract of *Terminalia chebula (TC) Tylophora indica (TI), Nycthathes arbor-tristis (NA)* and *Punica granatum (PG)* exhibited alleviated levels against OVA-specific IgG and IgM antibody responses as shown by enzyme-linked immune sorbent assay (ELISA). These plant sources have been further subjected to organic solvent extraction, chloroform (Chl), ethyl acetate (EA), and ethanol (Eth) for the purification of putative principles. The ethanolic fraction of these plants has been found to be having highest activity. The results obtained in this study shown that ethanol extracts of TC enhanced levels of IgG and IgM when compared to other solvent extracts.

KEY WORDS: **Immunomodulatory, *Terminalia chebula, Tylophora indica, Nycthathes arbor-tristis, Punica granatum,* ELISA.**

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INTRODUCTION

Plant sources have been used as medicine from the beginning of human civilization. India is a rich source of herbal medicine [1, 2]. In recent days, maximum numbers of plants are being screened for their potential pharmacological value. Various active compounds are isolated from plant sources which are found to be having different therapeutic applications. Ayurveda has been most popular since ancient times in India. The therapeutic regimens in ayurveda involve herbal sources as they have many ingredients which are helpful for alleviating ailments[3]. The most important of these plant phytochemicals are flavonoids, alkaloids, tannins, terpenoids, glycosides, phytosterols and phenolic compounds [4]. These compounds are responsible for multiple biological effects including immunomodulatory [5], antifungal and anti bacterial [6], antioxidant [7], anti-viral [8] anti-inflammatory activities [9] and anti-diabetic and anti-proliferative [10]. Immunomodulators are the substances which can stimulate, suppress or modulate immune system. The substance which suppresses immune system is immunosuppressants and which stimulates are immuno stimulators. There are different examples of suppressants such as Prednisolone,Cyclosporine, Tacrolimus, Sirolimus, Everolimus, Azathioprine, Mycophenolate, Mofetil, methotrexate, cyclophosphamide, thalidomide and chlorambucil , Interferon . Immuno stimulants commonly used are Levamisole, Thalidomide, BCG,Recombinant Cytokines, Interferons,Interleukin-2, inosiplex, azimexon, imexon, thymosin, methylinosine monophosphate, Vaccines, Immune Globulin, Rho (D) Immune Globulin[11] Immunosuppressants were mainly useful in auto immune diseases, grafting, transplant rejection where as immune modulators are mainly applicable in treatment of cancer and immune diseases[12]. In the present study ten different medicinally important plants were screened they are *Terminalia chebula, Punica granatum, Syzium jambolanum, Aegle marmelos, Nyctanthes arbor-tristis, Annona squamosa, Acalypha indica, Zea mays, Mimordica charantia, Tylophora indica* were selected to carry out immuno modulatory studies.

MATERIALS AND METHODS

(i) ANIMALS

Female Balb/c mice (6- 8 weeks old) were obtained from National Centre for laboratory Animal sciences (NCLAS), NIN, Hyderabad, Telangana, India. The animals were fed with food and water *ad libitium*, 12 hours of light and dark conditions were maintained. All the animal experiments were carried according to CPCSEA rules (GU/GIS/IAEC/2013/Protocol No.10/2013).

(ii) CHEMICALS

Ovalbumin (OVA), Ortho phenylenediamine (OPD), Goat anti-mouse IgG and IgM, Rabbit anti goat –HRP conjugate were procured from sigma aldrich, Mumbai, 96 well microtiter flat bottom Enzyme-Linked Immunosorbent Assay (ELISA) plates (Nunc, Denmark). All other chemicals were procured from local vendors and they are of analytical grade.

(iii) COLLECTION OF PLANT MATERIAL

Terminalia chebula (voucher number. BDH-22201 deposited in herbarium of Botany Department, Andhra university, visakhapatnam)*, Punica granatum*, *Syzium jambolanum, Aegle marmelos, Nyctanthes arbor-tristis, Annona squamosa, Acalypha indica, Mimordica charantia, Tylophora indica* plants were collected from Kakinada (Latitude-16^{o}93'N, Longitude-82^{o}33'E), East Godavari district, North Coastal region of Andhra Pradesh and were authenticated by botanist. Fresh seeds of *Zea mays* were obtained from local market.

(iv) PREPARATION OF PLANT EXTRACTS

Leaves of the plants and seeds of *Zea mays* were shade dried and pulverized in an electric grinder. The extracts were prepared using water and organic solvents like chloroform, ethanol and ethyl acetate. Aqueous extracts were concentrated by freeze dryer (Lark pengu classic plus) and the solvent extraction was done using soxhilation apparatus and concentrated using rotary evaporator (Superfit PBV-6).

(v) PROTOCOL AND DOSAGE FOR IMMUNIZATION

Mice were immunized on 0th, 21st, 42nd days intraperitonially with 0.5mL of volume of sample per mouse. The immunization protocol shown in figure.1 was followed in the present investigation[13]. Aqueous plant samples for immunization were prepared by dissolving 1mg of extract and 10µg of antigen (ovalbumin) in 0.5 ml of phosphate buffer saline (PBS). Organic fractions were dissolved in Dimethyl sulfoxide (DMSO). Mice were divided into different groups with 4 mice per each group[13]

(vi) COLLECTION OF SERUM

The mice were bled on 7th, 28th, 49th days after the immunization from the tail vein into a glass centrifuge tube and the blood was allowed to clot. The clotted blood was rimmed and the serum was separated after centrifugation at 8000 rpm (REMI R-8C) and stored at -20°C for further use.

(vii) EFFECT OF AQUEOUS AND SOLVENT EXTRACTS ON ANTI-OVA IgG AND IgM ANTIBODY LEVELS BY ELISA

A 100µl of antigen ovalbumin (200 ng) solution is coated to 96 well micro titre plates and incubated over night at 4°c. Plate was washed thrice with wash buffer (PBS-T) and wells were blocked with blocking agent (2% skimmed milk powder) and incubated for 2 hours at 37^{o}C. After washing, the plates were incubated with 100 µl of primary antibody (serum) incubated for 1 hr at 37^{o}C. Again washed thrice, then the plates were incubated with 100µl secondary conjugated antibody (Rabbit anti goat –HRP conjugate, 1000 dilution) for 1 hour at 37^{o}C. After washing, plates were incubated with 100 µl substrate solution (14mg OPD+H_2O_2/10ml) and then the reaction was stopped after 3 minutes by 50 µl of stop solution (8N

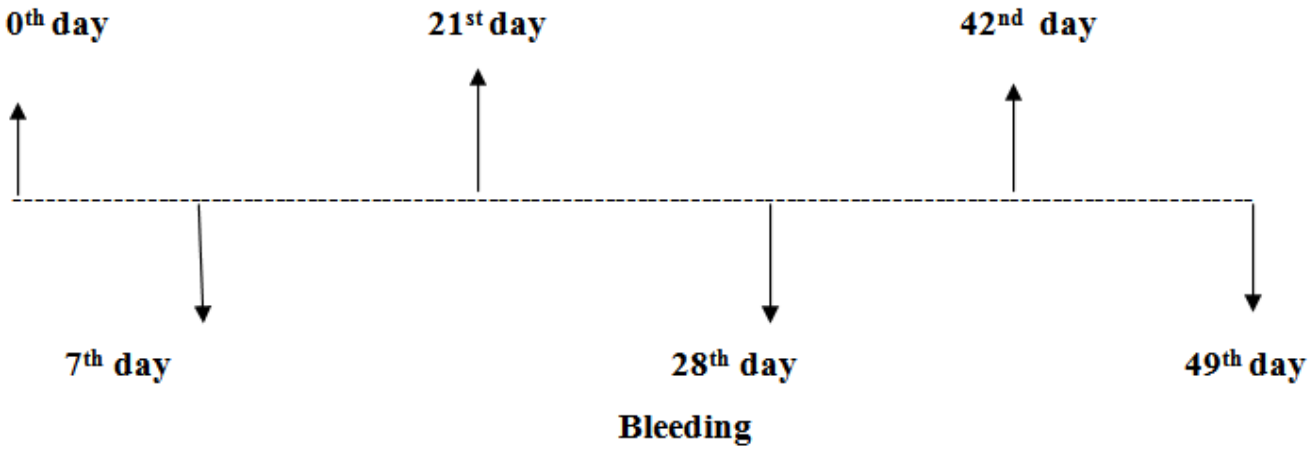
H_2SO_4). Optical density was measured at 492nm using ELISA reader (Thermo scientific Multi scan FC). The data expressed was the mean of Optical Density (OD) of the triplicates.

RESULTS AND DISCUSSION

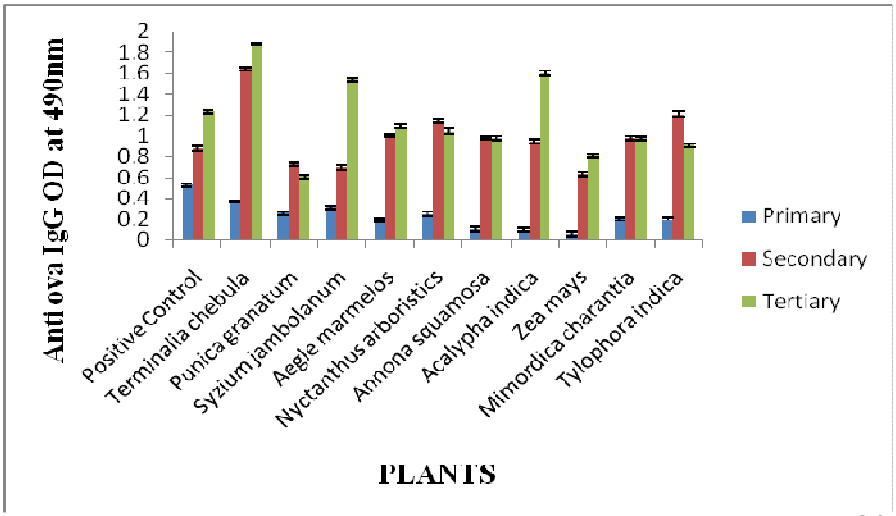
In the present study, out of the ten different medicinal plants selected, aqueous extract of TC has shown better immunostimulant activity, *Tylophora indica* (TI) showed moderate stimulant activity and least activity was shown by *Zea mays* (ZM) for anti ova Ig G (Graph 1), where as aqueous extracts of anti-ova IgM TC showed better activity, *Tylophora indica* (TI) showed moderate activity and ZM showed least immunostimulant activity (Graph 2). TC,PG,NA,TI showed enhanced immunostimulant activity for anti-ova IgG and anti-ova IgM, further TC,PG,NA,TI these plant extracts were fractionized using ethanol, ethyl acetate and chloroform. Ethanolic extract of TC shown highest stimulant activity for both antibodies IgG and IgM (Graph 3 & 4). Similar results have been reported by vaibhav *et al*., 2010 in TC dry ripe fruits. The solvent extracts of PG shown moderate stimulant activity against anti-ova IgM and TI against anti-ova IgG, whereas NA shown less stimulant activity when compared with positive control against both anti-ova IgG and IgM. Our results indicated that the ethanol fraction of TC enhanced ovalbumin induced IgG and IgM antibody response. It was revealed these active immune stimulant activities.

Immunization

Figure 1
Immunization protocol to determine the levels of anti-ova IgG and IgM in Balb/c mice



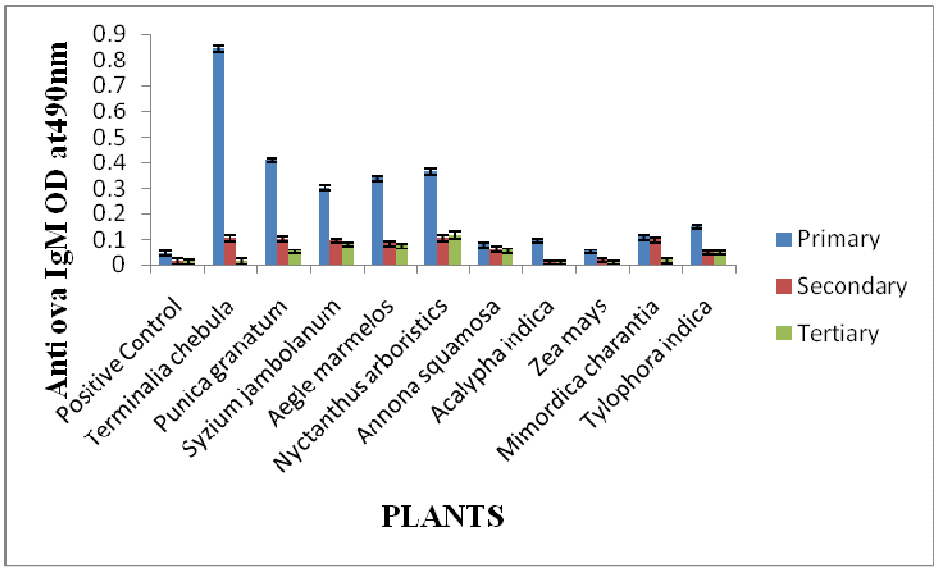
Graph 1
Effect of Plant extracts on anti-ova IgG response in Balb/c



Aqueous crude extract (1mg) was administered along with ova (10µg/mice) in 0.5 ml PBS for extracts on anti-OVA IgG response in Balb/c

Graph 2

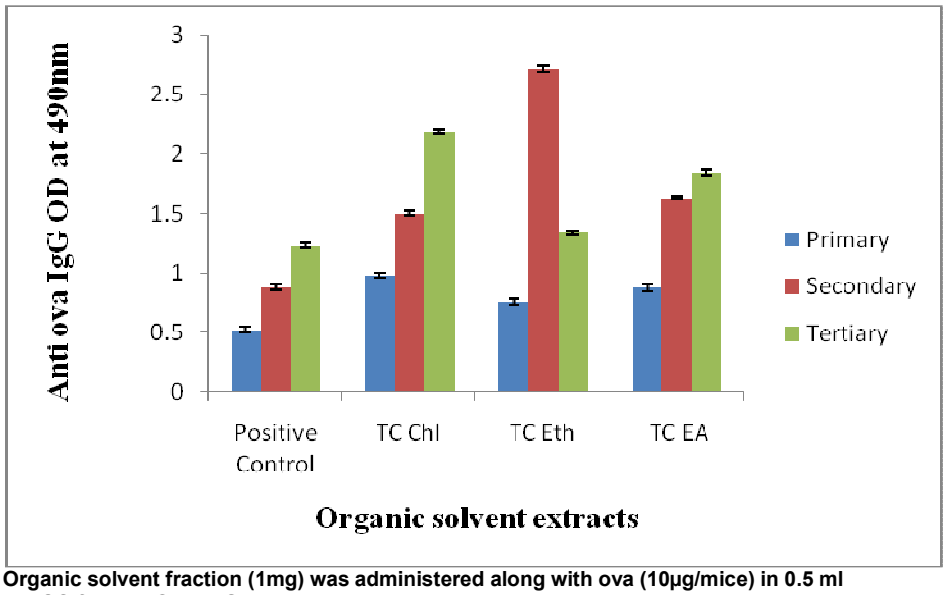
Effect of Plant extracts on anti-ova IgM response in Balb/c



Aqueous crude extract (1mg) was administered along with ova (10µg/mice) in 0.5 ml PBS for anti-OVA IgM response in Balb/c

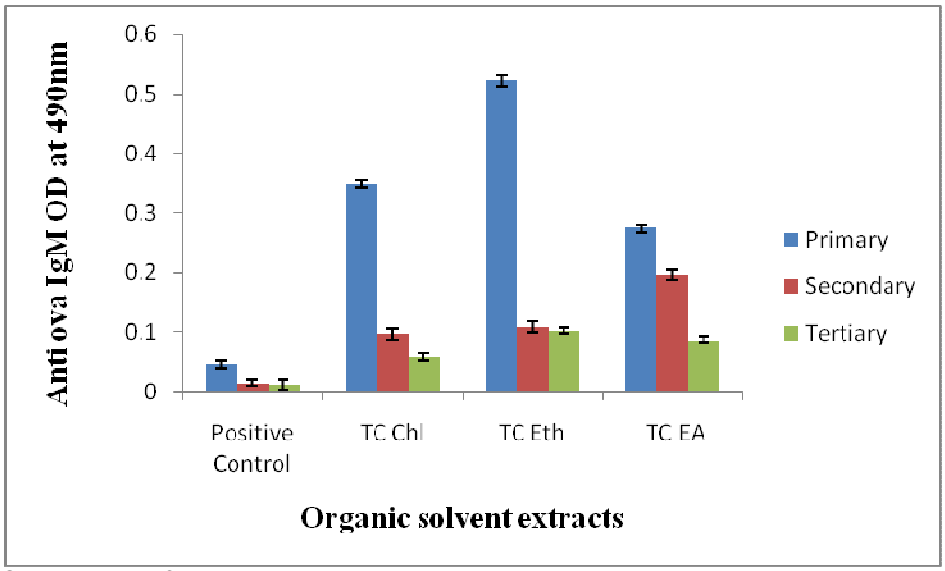
Graph 3

Effect of organic solvent fractions on anti-ova IgG response in Balb/c



Organic solvent fraction (1mg) was administered along with ova (10µg/mice) in 0.5 ml DMSO for anti-OVA IgG response in Balb/c

Graph 4
Effect of organic solvent fractions on anti-ova IgM response in Balb/c



Organic solvent fraction (1mg) was administered along with ova (10µg/mice) in 0.5 ml DMSO for anti-OVA IgM response in Balb/c

CONCLUSION

In the present investigation, 10 different medicinal plants were screened for immunomodulatory activity. Out of which, TC, TI, NA and PG shown better immunostimulant activity. Many substances produced by human body function as immune stimulants. For the sake of future research, immunostimulatory compounds have to be isolated and purified from different medicinal plants as they have their own importance in enhancing immune function.

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