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Research Article

Immunopharmacological activity of medicinal plants against *Aristolochia bracteolate* and *Phallus impudicus*

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ABSTRACT

Introduction: Immunological attempt was made to determine the potential of seven important medicinally plants viz., *Ficus benghalensis, Mangifera indica, Adhatoda vasica, Syzygium cumini, Terminalia arjuna, Ficus religiosa* and *Azadirachta indica* against protein antigen extracted from *Aristolochia bracteolate* (leaves) and *Phallus impudicus* (fungi).

Materials and Methods: Indirect ELISA was performed using crude protein as antigen extracted from *Aristolochia bracteolate* (leaves) and *Phallus impudicus* (fungi) and determined antibody production against aqueous leaves extract of seven different medicinal plants. In addition, in *vitro assay* was also performed using aqueous extract of active candidates in order to evaluate its inhibitory or stimulatory effect against these protein antigens.

Results: The result of these studies showed that *Azadirachta indica* (aqueous leaves extract) (followed by *Mangifera indica, Ficus religiosa* and *Terminalia arjuna*) showed higher antibody production and inhibition in proliferation rate (observed in lysed human whole blood) against protein antigen of *Phallus impudicus* and *Aristolochia bracteolate* effect as compared to seven different medicinal plants.

Conclusion: Out of these results it is confirmed that these medicinal plants especially *Azadirachta indica* showed antifungal and anti-inflammatory effect against protein antigen of *Phallus impudicus* and *Aristolochia bracteolate*.

Keywords: *Ficus benghalensis; Mangifera indica; Adhatoda vasica; Syzygium cumini; Terminalia arjuna; Ficus religiosa; Azadirachta indica; Immunosuppressive; ELISA*

Introduction:

Medicinal plants are generally used in many countries for various purposes (e.g. starting material for drug preparation) and considered to be rich source of potent drugs against various infectious agents [1, 2]. Immunobiological activity of these medicinal plant products that are beneficial to human including animals are due to the presence of primary and secondary metabolites with potential therapeutic effects [3]. Traditionally, these medicinal plant products are more acceptable and considered to be a part of human health care [3, 4]. Mostly people from Asian countries are generally believed in natural medicines as compared to modern synthetic drugs. In this study, we focused on various medicinal plants and tried to determine its effect against protein antigen extracted from *Aristolochia bracteolate* (leaves) and *Phallus impudicus* (fungi).

Traditionally, whole plant of *Ficus benghalensis* (Vad; family *Moraceae*) medicinal plant that is reported as anti-oxidant, anti-cancer, anti-microbial properties and also used in the treatment of several diseases [5-7] e.g. skin, vaginal disorders etc. *Mangifera indica* (Mango; family *Anacardiaceae*) displayed various medicinal uses and treat number of diseases including viruses and bacteria [8]. Number of polyphenolic compounds e.g. Mangiferin are reported in leaves aqueous extract of *Mangifera indica* [8, 9]. *Adhatoda vasica* (Adulsa; family *Acanthaceae*) are normally used for various infectious diseases e.g. asthma and bronchitis patients [10]. *Syzygium cumini* (Jamun; family *Myrtaceae*), medicinal plant

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are commonly grown in subcontinents including India and showed various immunopharmacological activities such as antidiabetic, antioxidant, anti-inflammatory, gastroprotective etc.[11] *Terminalia arjuna* (Arjuna; family *Combretaceae*), medicinal plant and the main constituents that are reported (i.e. tannins, arjunic acid; arjunolic acid; arjungenin; luteolin, gallic acid, ellagic acidetc.) and responsible for various activities [12, 13]. *Ficus religiosa* (Peepal; family *Moraceae*), medicinal plant and is used for curing various diseases e.g. gastric problems and inflammatory disorders [14]. *Azadirachta indica* (neem; family *Meliaceae*) extracts or fraction have vast immunopharmacological activities (anti-inflammatory, antimicrobial etc.) and reported several biologically active molecules such azadirachitin [15].

Aristolochia bracteolate (worm killer; family *Aristolochiaceae*) medicinal plant and is used for various inflammatory (arthritis) and cardiovascular (diabetes) diseases and also showed immunopharmacological activities i.e. antipyretic, antimicrobial etc. [16-18] whereas *Phallus impudicus* (family *Phallaceae*) identified through tip (at the top with foul smelling) and volatile compounds are identified e.g. mature fruit bodies (Dimethyl trisulfide, cis-β-ocimene, trans-β-ocimene, 2-phenylacetladehyde and 2-phenylethanol) etc. [19-21] The main purpose of our study is to observe its immunological effect of protein extracted from *Aristolochia bracteolate* (leaves) and *Phallus impudicus* (fungi) whether this protein showed some additive or synergistic or immunosuppressive effect after treating with variable concentration of aqueous leaves extract of various medicinal plants.

MATERIALS AND METHODS

Plant material

Leaves of various medicinal plants i.e. *Ficus benghalensis, Mangifera indica, Adhatoda vasica, Syzygium cumini, Terminalia arjuna, Ficus religiosa* and *Azadirachta indica* were collected from Nakshatra Udyan, Baramati region, Maharashtra, India.

Preparation of extracts

Leaves of various plant materials (as mentioned above) were washed thoroughly under tap water and dried in a shady area and then macerated in mortar and pestle using liquid nitrogen to prepare finely powder form. Aqueous plant extracts of plant material was exhaustively extracted by mixing 5 gm of powdered plant material and adding approximately 50ml of phosphate buffered saline (PBS, pH 7.2) and was allowed to macerate at room temperature for 10-15 minutes and then extract was filtered through filter paper. Collect the filtrate and performed various immunological studies.

Collection of samples for protein extraction

Both the species i.e. *Aristolochia bracteolate* (leaves) and *Phallus impudicus* (stinkhorn, fungi) were collected. The plant material and fungus was identified by Dr. Bharat Shinde, Principal Vidya Pratishthan Arts, Science and Commerce College, Vidyanagari Baramati. After identification, this sample was used for immunological studies.

Analysis of protein content through SDS PAGE

In SDS PAGE (resolving, 10 % and stacking, 8% gels) were used for identification of protein bands in *Phallus impudicus* and *Aristolochia bracteolate*. In this study, both the samples (50 µl) were loaded into the wells and current of 15 mAh for stacking gel and 25 mAh for separating gel was required to run the gel. After the separation of protein bands through electrophoresis, staining solution was utilized to stain the gel in order to make bands visible. Afterwards the gel was placed in to a destaining solution for 24 hours on shaker and was changed frequently until clear gel was obtained.

ELISA

Indirect Elisa was performed using these two proteins as coating antigen [6.4 mg/ml; 100 µg/well and 4 mg/ml; 100 µl) of *Aristolochia bracteolate* and *Phallus impudicus*. Aqueous leaves extract of seven different materials were tested and determined antibody (IgG) titre against these two fungal species. Horse anti-serum used as secondary antibody and optical density measured at450 nm [15].

In vitro experiment (proliferation assay)

In vitro study was employed for the evaluation of protein extracted from *Phallus impudicus* and *Aristolochia bracteolate* along with variable doses of active candidate of plant material (based on

ELISA results) on lysed human whole blood for determining its immunological activity. The study was conducted at different dilutions of active candidates of aqueous extracts (final volume i.e. 50 µl) prepared in PBS on human lysed whole blood (10^5 cells/well; 100 µl) along with mitogen Concanavalin A (Con A, 2.5 mg/ml; 10 µl) and protein content of *Phallus impudicus* and *Aristolochia bracteolate*. The crude protein was diluted in PBS and PBS alone served as negative control. Incubate plate for 24 h and then proceed for MTT proliferation assay [6-9]. After incubation, add MTT dye (2.5 mg/ml; 10 µl) and again incubate plate for another 4h. The formazan crystals settled at the bottom after centrifuging and discard the supernatant. Dissolve formazan crystals with dimethyl sulphoxide (DMSO) solution and optical density were measured at 570 nm.

RESULTS

SDS PAGE

The results showed that presence of two prominent bands of around 30 - 40 KDa and 100 KDa as shown in **Fig.1.**

ELISA

As shown in **Fig.2**, the results showed that *Azadirachta indica* showed higher antibody (IgG) production against these two proteins extracted from *Phallus impudicus* and *Aristolochia bracteolate.* In this study, seven medicinal plants were selected and antibody production against *Aristolochia bracteolate* followed in this range i.e. *Azadirachta indica > Mangifera indica > Ficus religiosa > Adhatoda vasica > Terminalia arjuna* where as in case of *Phallus impudicus, Azadirachta indica > Mangifera indica > Ficus religiosa.*

In vitro experiment

As shown in **Fig.3**, the results showed that *Azadirachta indica* showed higher inhibitory effect in comparison with rest of four other medicinal plants. The pattern of inhibitory activity will be observed in this order i.e. *Azadirachta indica > Mangifera indica > Ficus religiosa > Terminalia arjuna.*

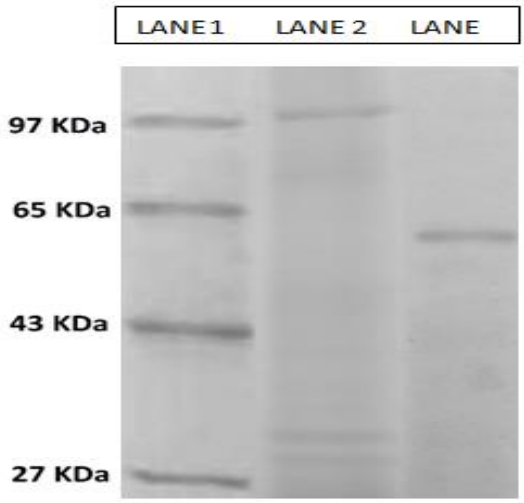
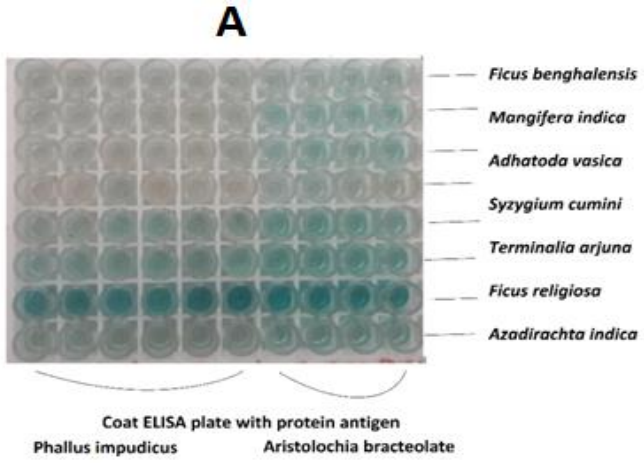


Figure 1: SDS-PAGE analysis of crude protein isolated from fungi (*Phallus impudicus*) and aqueous leaves extract of *Aristolochia bracteolate*

LANE 1- HMW (high molecular weight) Protein marker

LANE 2- Protein (*Aristolochia bracteolate)*

LANE 3- Protein (*Phallus impudicus*)



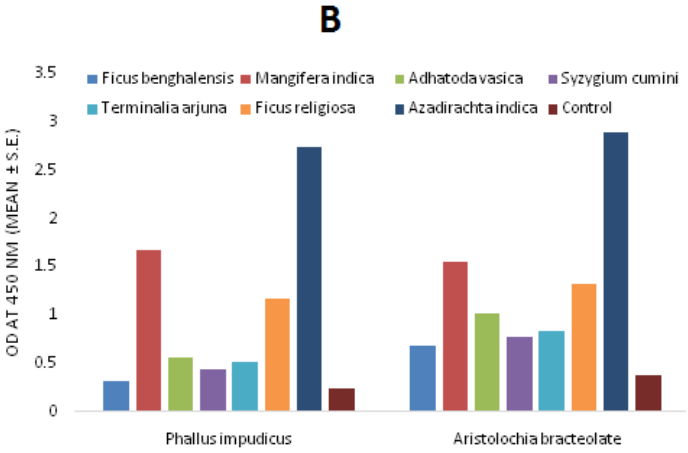
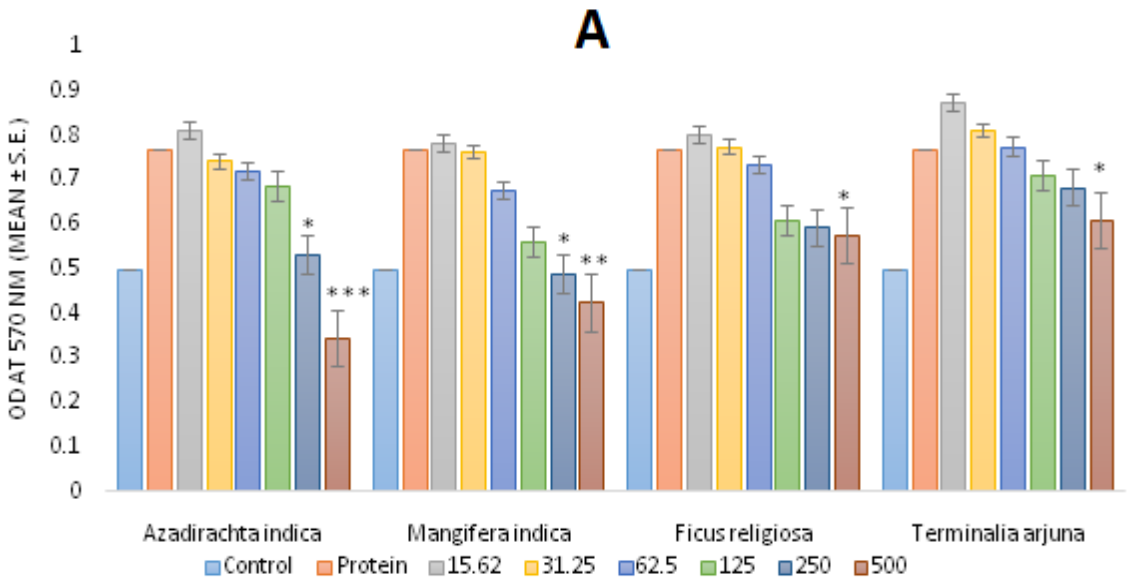


Figure 2 (A) (B): ELISA assay. Indirect Elisa was performed using *Aristolochia bracteolate* (6.4 mg/ml; 100 µg/well) and *Phallus impudicus* (4 mg/ml; 100 µl) as coating antigen. Aqueous extract of various medicinal plants (as mentioned in materials and methods section) were used for the estimation of antibody production against protein antigen of both the species. Horse anti-serum used as secondary antibody and optical density measured at 450 nm.

Aristolochia bracteolate



Phallus impudicus

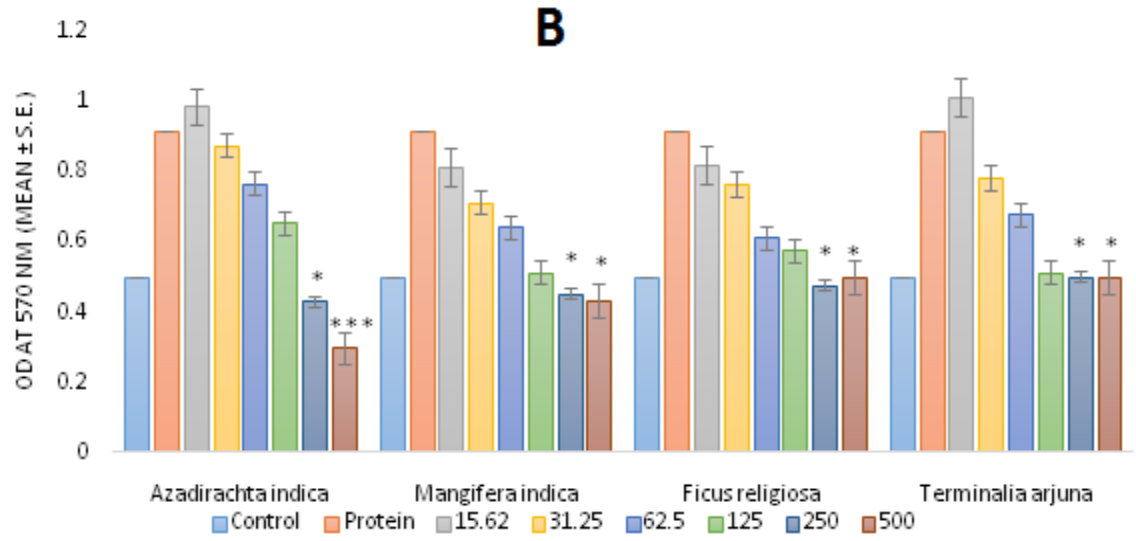


Figure 3 (A) (B): Proliferation assay. Lysed human whole blood was cultured for 24h along with Con A along with variable doses of aqueous leaves extract of various medicinal plants and protein (as described in materials and methods section). After incubation, proliferation was measured by MTT assay. The results are presented as Mean ± S.E. The difference between control, standard and aqueous leaves extract is determined through one way ANOVA test. *P* values: **P < 0.05, **P < 0.01*

DISCUSSION

Mostly infections are generally targeted to animals and human through some carrier molecule or agent but it is very difficult to diagnose it. Some of the diseases are generally caused by some specific proteins from pathogens [15]. In this study, we focused on various medicinal plants in the form of aqueous leaves extract against protein antigen extracted from *Phallus impudicus* and *Aristolochia bracteolate* and determined various immunological assays. From these studies, we observed that aqueous leaves extract of *Azadirachta indica* exhibited higher antibody production against both the protein antigens but it showed inhibitory activity at higher concentration against protein antigen of *Phallus impudicus* and *Aristolochia bracteolate*. So, these results suggest that *Azadirachta indica* could be a potential source of antifungal compounds against protein of *Phallus impudicus* where as in *Aristolochia bracteolate,* aqueous leaves extract of *Azadirachta indica* showed some immunosuppressive effect after treating with protein antigen of *Aristolochia bracteolate* so it may showed some inhibitory effect as well. So, the interaction between aqueous leaves extract containing various metabolites and antigen-specific protein extracted from fungus (*Phallus impudicus)* and leaves (*Aristolochia bracteolate*) provides important signals for the efficient activation or inhibition of T cell response.

In this study, lysed human whole blood was cultured with Con A for T cell activation. From these studies, we concluded that aqueous leaves extract of *Azadirachta indica* followed by *Mangifera indica, Ficus religiosa* and *Terminalia arjuna* showed dose-dependently decreased in proliferation rate against protein antigen of *Phallus impudicus* and *Aristolochia bracteolate*. On the other hand, these aqueous leaves extract especially *Azadirachta indica* showed immunosuppressive or anti-inflammatory effect at much higher concentrations. In addition, this effect is also due to the presence of protein (determined through SDS PAGE) i.e. approx. 60 kDa (*Phallus impudicus*) and 100 kDa (*Aristolochia bracteolate*). Normally, 60 kDa protein is associated with protection against stress conditions [22] whereas 100 kDa represents aristolochic acids may regulate immune responses and should be attractive candidates for immunosuppressive activity [23].Therefore, direct exposure of these aqueous leaves extract of active candidate especially *Azadirachta indica* on lysed human whole blood can be considered more suitable method for evaluation purposes of immunomodulatory agents.

Modulation of immune response using various medicinal plant products against protein antigen of both the species in human whole blood pertaining to determined its immune-pharmacological activity. The results of this study which clearly indicates its antifungal effect of A*zadirachta indica* against protein antigen of fungus (*Phallus impudicus) i.e.* decline in proliferation but enhancement in antibody production and also showed immunosuppressive or anti-inflammatory effect against protein antigen of *Aristolochia bracteolate.* Overall, the data showed that these medicinal plants especially A*zadirachta indica* showed immunosuppressive or anti-inflammatory and antifungal effect.

CONCLUSION

The effect of various medicinal plants on protein antigen extracted from fungi (*Phallus impudicus)* and leaves (*Aristolochia bracteolate*) which clearly indicate that these phytochemicals may have the capability to enhance antibody production against these protein antigens and also showed some suppressive effect as well. In view of these observations further immunobiochemical studies involving isolated active component of these medicinal plants are warranted to confirm its immunological activity.

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