



# Immunopharmacological screening of aqueous root extract of *Santalum album*

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## ABSTRACT

**Introduction:** In order to scrutinize the anti-microbial and anti-inflammatory agents, many research groups have focused their works on the field of immunopharmacology. Thus, the current study aimed to evaluate the immunopharmacological activity of aqueous root extract of *Santalum album* against Hepatitis B surface antigen (HBsAg) and Newcastle disease virus (NDV) on human peripheral blood mononuclear cells (PBMCs).

**Methods:** Variable doses of aqueous root extract of *Santalum album* (0.5-30 mg/ml, 50 µl) were prepared using phosphate buffered saline (PBS) and tested for proliferation assay, nitric oxide production and CD14 monocyte surface marker using flow cytometry.

**Results:** Aqueous root extract of *Santalum album* inhibited proliferation, nitric oxide production and CD14 monocyte surface marker at higher doses.

**Conclusion:** Aqueous root extract of *Santalum album* possesses both anti-inflammatory as well as antiviral activities and might be used for these purposes.

### Implication for health policy/practice/research/medical education:

*Santalum album* has anti-inflammatory and antiviral activities and might be used for these purposes.

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## Introduction

Medicinal plants have been continuously used for thousands of years to treat various disorders and to prevent a number of animal and human diseases (bacteria, viruses, fungi, etc.) (1,2). The knowledge of various medicinal plant properties has been transmitted all over the world. It has been reported that the active constituent is responsible for immunopharmacological properties of medicinal plants so that some of them are continuously used throughout the world for different purposes (3,4).

There are a large number of medicinal plant products, potentially able to control various diseases and also used for specific case of disease treatment (1). For the last twenty years, lot of work was done on many medicinal plant products with description of their chemical constituents in the form of primary and secondary metabolites (2,3). These metabolites could be isolated using various types of qualitative and quantitative based assays, leading to understand the mechanism and correlation between these metabolites

and disease model studies (5). Thus, the present work was focused on *Santalum album*, as one of medicinal plants, with emphasis on its antimicrobial properties in human peripheral blood mononuclear cells (PBMCs).

Newcastle disease virus (NDV, single-stranded RNA virus) belongs to the family Paramyxoviridae and genus *Aulavirus* with helical capsid symmetry. Till now, NDV disease still burdens for the poultry industry with several consequences including an increase in mortality rates (5). The only control method for NDV disease is through vaccination but it does not provide hundred percent protection. Thus there is a need to search for those medicinal plants to control the burden of NDV disease.

One of the medicinal plant, *Santalum album* (commonly known as chandan) in the family *Santalaceae*, is usually found in India, china, Indonesia and Philippines (6,7). *Santalum album*, is a widely plant commonly found in India especially parts of Karnataka and adjoining districts of Maharashtra, Tamil Nadu and Andhra Pradesh in India.

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Actually, this plant is being exploited for various purposes especially sandalwood oil is obtained mostly by steam distillation of its root and heartwood (8,9) with a number of medicinal uses e.g. treatment of many other ailments like diarrhea along with bleeding, intrinsic hemorrhage, inflamed hemorrhoids (piles), eye infections, inflammation of the umbilicus, hiccoughs initial phase of pox, and urticaria (10,11). The current study was focused on the immunopharmacological activity of *Santalum album* on human PBMCs against Hepatitis B surface antigen (HBsAg) and NDV.

## Materials and methods

### Assemblage and composition of plant material

The fresh roots of *Santalum album* were collected from the garden of Vidya Pratishthan's in the morning, between January and February 2015 in Baramati region, District Pune, Maharashtra, India. Afterwards, the fresh roots were washed in tap water and then with distilled water to remove dust and finally dried in a shady area. The plant roots were macerated with liquid nitrogen to prepare a fine powder. This powder was used for subsequent immunopharmacological assays.

### Phytochemical screening and extraction

Different qualitative and quantitative based assays were carried out in order to determine the presence of secondary metabolites. Qualitative based assays revealed aqueous root extracts of *Santalum album* and the presence of Saponin (foam test); terpenoids (acetic anhydride test) and flavonoids (alkaline reagent test).

### Collection of NDV samples

NDV Samples of suspected birds were collected under the programme of Biovillage scheme, Vidya Pratishthan's School of Biotechnology.

Specific pathogen free chicken eggs were purchased from Venkys India Ltd. The allantoic cavity route of embryonated (9-11 day old) chicken eggs was used for isolation and propagation of NDV from field samples (5). These eggs were observed in dark (using candle) at regular intervals and bigger sized embryos (air cell and area without blood vessels) selected for inoculation. After disinfection of egg shells with spirit, 0.2 ml of supernatant was inoculated at 45° angle into embryonated chicken eggs. Embryo motility was observed every 10 hours by candling. After the death of embryos, amnio-allantoic fluid was harvested and checked for presence of virus. This was determined through haemagglutination test (128 HA titre value).

### PBMC proliferation assay and nitric oxide production using HBsAg and NDV

EDTA anti-coagulant human blood samples were collected from Mangal Pathology Laboratory, Maharashtra, India and scrutinized at the VSBT, Baramati, Maharashtra, India, between January to February 2015.

PBMCs were separated by means of density gradient centrifugation and cultured with variable doses of aqueous root extracts (0.5-30 mg/ml, 50 µl) of *Santalum album* along with or without HBsAg (20 µg/ml, 10 µl) and NDV (1:80 dilution, 10 µl). Incubate 96-well plates for 48 hours at 37°C. HBsAg and NDV used as standard for these studies. Centrifuging the plates was done at 2500 rpm for 10 minutes at 4°C with collecting the supernatant for the estimation of nitric oxide production through Griess reagent. Afterwards, fresh complete medium was added into the 96-well plates. Again, incubating the plates for another 4 hours along with (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide; 5 mg/ml, 10 µl) continued. After incubation, the plates were suddenly centrifuged with discarding the supernatant, collecting the pellet and finally dispersing in dimethyl sulphoxide (DMSO) solution. The optical density was measured at 570 nm (12,13). After preincubation of PBMC (10<sup>5</sup> cells/ml) with or without HBsAg and NDV for 48 hours the supernatant (as mentioned above) was collected for estimation of nitric oxide production. Briefly, 50 µl of untreated and treated PBMC cell culture medium along with HBsAg/NDV was assorted with 50 µl of Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride [NED] in 2.5% phosphoric acid). For this experiment, Roswell Park Memorial Institute medium (RPMI) containing 10% fetal bovine serum was used as a blank followed by incubation of 96-well plates at room temperature for 10-15 minutes. After incubation, absorbance at 540 nm was measured in a microplate reader (14,15).

### Estimation of CD14 monocyte surface marker by flow cytometry

In another set of experiments, human PBMCs were cultured with serial dilutions of aqueous root extracts of *Santalum album* along with or without HBsAg (20 µg/ml, 10 µl)/NDV (1:80 dilution, 10 µl) for 48 hours in 96-well plates. After incubation, samples of the PBMCs treated with or without HBsAg/NDV were collected and stained with CD14 FITC (3 µl) monoclonal antibody. The samples were then incubated, lysed and washed with phosphate buffered saline (PBS). Finally, the samples were prepared for flow cytometric analysis. The resulting stained cell pellet was resuspended in 2000 µl of PBS and run on a FACS Calibur flow cytometer (16,17).

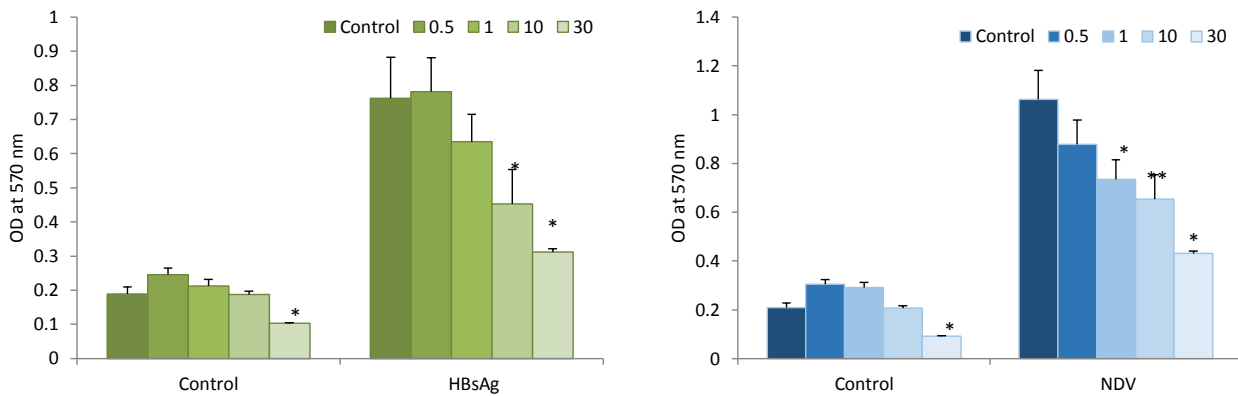
### Statistical analysis

All values were mentioned as mean ± SE. Data was represented by one-way analysis of variance (ANOVA) test (Bonferroni multiple comparison test).

## Results

### PBMC proliferation assay

The effect of variable doses of aqueous root extract of *Santalum album* on PBMC proliferation assay with or without HBsAg and NDV using MTT is shown in Figure 1. The



**Figure 1. Effect of variable doses of aqueous root extract of *Santalum album* on HBsAg and NDV in human PBMC.** PBMC were cultured with or without optimized dose of HBsAg and NDV along with variable doses of aqueous root extract (0.5, 1, 10 and 30 mg/ml, 50  $\mu$ l) or NDV/HBsAg alone. After 3 days, proliferation was measured by MTT assay. The results are presented as Mean  $\pm$  S.E. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001 as compared to control.

aqueous extract showed a dose-dependent decrease in the proliferation assay with or without HBsAg/NDV as compared to control. Also, there was a significant enhancement of PBMC proliferation as compared to control.

#### Nitric oxide estimation

The observed effect of variable doses of aqueous root extract in the cell culture medium of PBMC along with or without HBsAg/NDV is shown in Figure 2. The aqueous root extract showed a significant decrease in nitric oxide production at higher doses with or without HBsAg/NDV as compared to control. There was a significant enhancement of nitric oxide production as compared to control.

#### CD14 monocyte surface marker

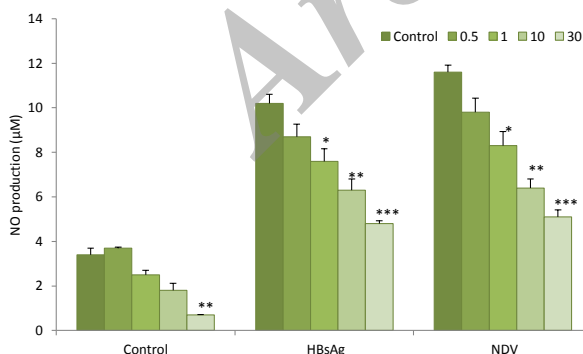
The effect of aqueous root extract of CD14 monocyte surface marker on PBMC with or without HBsAg/NDV is shown in Figure 3. There was a significant decrease in CD14 monocyte surface marker at higher doses as com-

pared to control.

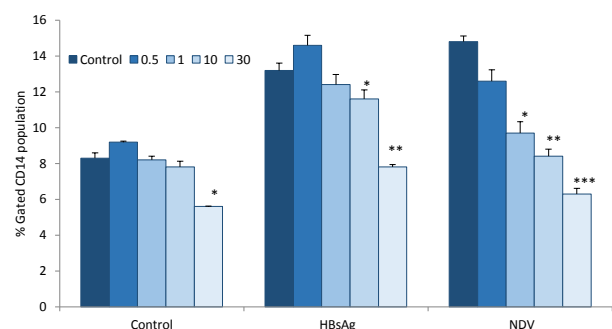
#### Discussion

The prime objective of our study was to investigate the aqueous root extract of *Santalum album* for in vitro proliferation, nitric oxide production and CD14 monocyte surface marker on human PBMC using HBsAg and NDV, in order to validate the potential use of this aqueous root extract as a tool in screening for anti-inflammatory and anti-microbial effects on the immune system.

As per the literature survey, there are a number of immunopharmacological studies related to medicinal plants which have already been done using non specific antigens on humoral and cell mediated immune response (18). These studies are a matter of great interest for many immunopharmacologists. For instance, one of the medicinal plant namely, *Santalum album* shows anti-inflammatory (reduction in HBsAg) as well as anti-microbial (inhibitory effect of NDV) activities at higher doses as compared to



**Figure 2. Estimation of nitric oxide production (NO) from human PBMC.** The supernatant nitrite concentration was determined by Griess reagent after 48 h culture of cells in presence of with or without HBsAg and NDV along with variable doses of aqueous root extract (0.5, 1, 10 and 30 mg/ml, 50  $\mu$ l) or NDV/HBsAg alone. Values are expressed as Means  $\pm$  S.E. The difference between the control and treated groups is determined by One-way ANOVA test (Bonferroni multiple comparison test). \* $P$  < 0.05; \*\* $P$  < 0.01; \*\*\* $P$  < 0.001.



**Figure 3. Flow cytometric analysis of aqueous root extract extracted from *Santalum album* on CD14 monocyte surface marker.** PBMC were treated with variable doses of aqueous root extract (0.5, 1, 10 and 30 mg/ml, 50  $\mu$ l) along with or without NDV/HBsAg and then lysed and wash the cells with PBS and analyzed through flow cytometer (FACS, Calibur) using forward and side scatter gating applied for data acquisition of 10000 events of cell populations representing different phenotypes analyzed using cell quest software.

control and standard HBsAg/NDV.

One of the parameters interconnected with our immune system i.e. nitric oxide production, provides reliable information whether the aqueous root extract shows inhibitory or stimulatory effect with respect to PBMC proliferation assay and CD14 monocyte surface marker. The criteria for selecting this parameter i.e. nitric oxide (as a fundamental signaling agent), regulates various immunopharmacological functions and serves as a potent mediator of inflammation, bacteria or viral disease burden and cytotoxicity (14,15). The findings of the preliminary phytochemical investigations of the aqueous root extract of *Santalum album* and the results of anti-inflammatory activity against HBsAg are shown in the respective figures with anti-viral activity against NDV. The results indicate that aqueous root extraction of *Santalum album* shows a dosage-dependent relationship.

The capacity to elicit a decline in T cell immunity can be shown by the CD14 monocyte surface marker. This is determined through flow cytometry and generally used for measuring the properties of the cell suspended in a stream of fluid when passing through one, two or three lasers. It is also used for measuring the immunophenotyping i.e. detection of cell surface molecules such as cluster of differentiation (CD), cell cycle analysis, cell viability, total protein, enzyme activity, gene expression etc. (16,17). The results indicate that aqueous root extract could significantly reduce the CD14 count in human PBMC exposed to HBsAg and NDV. The results of our immunopharmacological studies on human PBMC after exposing with HBsAg and NDV suggest that the aqueous root extract shows anti-inflammatory as well as anti-viral effect on human PBMC.

### Conclusion

This study suggests that the aqueous root extract of *Santalum album* significantly inhibits the production of proliferation assay, nitric oxide production and CD14 monocyte surface marker when using HBsAg and NDV. Further investigations of the aqueous root extract should be done through in vivo assessment for immunopharmacological studies in mice models with identification of the major active components responsible for anti-microbial and anti-inflammatory activities.

### Acknowledgements

We would also like to thanks Dr. H. Pawar Veterinary Doctor for collection of NDV samples under the project of Biovillage-Sustainable development through Biotechnology.

### Authors' contributions

This work was carried out in collaboration between AG and SRC. AG anchored the field study, collected the data and prepared the manuscript. AG and SRC managed the literature searches and produced the initial draft. Both the authors read and approved the final manuscript.

### Conflict of interests

None to be declared.

### Ethical considerations

All these studies were conducted under surveillance of animal diseases in Baramati taluka which is ethically approved from IBSC committee, Savitri Phule University Pune, India.

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