

## Antimicrobial studies of *Ficus benghalensis* and *Ficus racemosa* on pathogenic viral diseases

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### ABSTRACT

**Background and aims:** Medicinal plant products are considered to be an effective candidate against the number of viral diseases as generally observed or reported in developing countries. As per the literature, secondary metabolites (i.e. Alkaloids, flavonoids, saponins etc.) are reported in medicinal plant products and showed its antiviral properties. In this study, our group focused on those medicinal plants especially roots of *Ficus benghalensis* and *Ficus racemosa* related to New castle Disease Virus (NDV) and Infectious Bursal Disease (IBD) having *in vitro* antiviral activity. These studies were conducted on the human peripheral blood mononuclear cells (PBMC).

**Methods:** For antimicrobial studies, different medicinal plant products especially roots of *Ficus benghalensis* and *Ficus racemosa* were collected from Vidya Pratishthan's garden, School of Biotechnology, Baramati. These medicinal plant leaves are used in the form of aqueous extract and determined its anti-microbial activity against poultry viruses (i.e. NDV and IBD) in PBMC and determined proliferation assay, Th1 (TNF alpha) cytokine production and CD14 monocyte surface marker.

**Results:** Three medicinal plant aqueous extracts showed noteworthy antimicrobial activity with respect to decline in proliferation assay, TNF alpha production and CD14 monocyte surface marker in human PBMC as compared to control.

**Conclusion:** Some of the medicinal plant products have shown antimicrobial activity. Further immunobiological research is mandatory to elucidate the most active constituents that are present in these aqueous extracts which may be useful or target molecule in the development of new effective and safe antiviral agents.

**Keywords:** *Ficus benghalensis*, *Ficus racemose*, Antimicrobial; NDV; IBD.

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### INTRODUCTION

One of the most important poultry viral diseases i.e. NDV and IBD causes enormous

or devastating losses in both commercial and village chickens. In an effort to reduce the

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burden of NDV and IBD disease in chickens, it becomes a critical step to upgrade their productivity.<sup>1,2</sup> In addition, NDV (family *Paramyxoviridae*) is contained in one serotype and therefore called as avian paramyxovirus serotype-1 (APMV-1) where as IBD virus (family, *Birnaviridae* of RNA viruses) especially serotype 1 is pathogenic and highly resistant to most of these disinfectants including environmental conditions.<sup>3,4</sup> The incubation period of IBD virus is short and the first symptoms will be appeared after 2-3 days infection. In the beginning of IBD disease, the bursa is enlarged, oedematous and covered with a gelatinous transudate whereas virulent isolates of NDV disease can result in rapid, high mortality of birds.<sup>5,6</sup> In an effort to reduce the burden of these poultry viruses it was used various medicinal plants because most of these antimicrobials are derived from medicinal plant products especially leaves, flowers, fruits, bark or from microorganisms.<sup>7,8</sup>

Though, antimicrobial properties of these medicinal plant products have been scrutinized time to time through various biological studies worldwide, but recently it has gained much more importance globally after the development of immunopharmacology.<sup>9</sup> Out of these medicinal plants, *Ficus benghalensis* and *Ficus racemosa* were selected for antimicrobial studies because of number of immunopharmacological activities e.g. anti-inflammatory, anti-oxidant, immunomodulatory, proteases etc.<sup>10-12</sup> Thus, in the present study, aqueous root extract of *Ficus benghalensis* and *Ficus racemosa* has been investigated for antimicrobial activities against NDV and IBD.

## METHODS

Roots of *Ficus benghalensis* and *Ficus racemosa* were collected from

Vidya Pratishthan's garden, Baramati, India. These fresh roots (10 g) were dried in a shady area and macerated in liquid nitrogen for 2-3 minutes with occasional stirring to prepare fine powder. Then the powder was macerated and dissolved in phosphate buffered saline (PBS, 100 ml). Thereafter, the filtrate was collected after centrifuging (10,000 rpm; 10 minutes) and studied its various immunopharmacological activities including qualitative analysis of secondary metabolites. Different assays were performed and revealed the presence of flavonoids (alkaline reagent test); alkaloids (Hager's test); terpenoids (using alcohol and petroleum ether) and saponin (foam test).<sup>9,13</sup>

Poultry disease samples (NDV and IBD) of suspected birds were collected aseptically under Biovillage programme scheme, Vidya Pratishthan's School of Biotechnology, Baramati, India. All these studies were conducted under IBSC guidelines and approved by Savitri Phule Pune University, Maharashtra, India.

For collection of NDV disease samples, pooled tissues (i.e. lung, spleen, intestine etc.) of suspected dead birds were collected, macerated and finally dissolved in PBS containing penicillin and streptomycin. Thereafter, supernatant of pooled tissues were collected after centrifuging (5000 rpm, 10 minutes). In this study, supernatant (0.2 ml) for detection of NDV was directly injected into the allantoic cavity route of chicken eggs (embryonated, 9-11 day old; Venkys India Ltd) that are totally pathogenic free. For inoculation, bigger sized embryos were selected and then continually observed at different time intervals in order to observe its embryo motility rate. For NDV confirmation, amnio-allantoic cavity fluid was collected and determined HA [128 titre] test.<sup>2,5</sup>

For IBD confirmation, bursal samples of poultry animals were macerated using

mortar and pestle and finally dissolved in PBS containing penicillin and streptomycin. These incubated bursal samples for 1-2 h and shake slowly every 5 minutes. Thereafter, supernatant was collected after centrifugation and inoculated into sterile blood agar media for bacteriological sterility and was incubated at 37 °C for 24 h. This sterile suspension (bacteriologically) was used as inoculums for the isolation of virus.<sup>3,6</sup>

EDTA blood samples of human (n= 5) were collected (*Mangal Pathology Laboratory*, Maharashtra, India) and separated PBMC through density gradient centrifugation.

In this assay, PBMC ( $10^5$  cells/well; 100  $\mu$ l) were cultured in 96 well plate for 48 h incubation along with variable doses of aqueous root extract (0.5-30 mg/ml, 50  $\mu$ l) of *Ficus benghalensis* and *Ficus racemosa* along with NDV (1:80 dilution, 10  $\mu$ l) or IBD (1: 100 dilution, 10  $\mu$ l). Thereafter, centrifuging the plates and collect supernatant for estimation of Th1 (TNF alpha) cytokine production. For proliferation assay, fresh complete media (containing penicillin, Himedia, code TC187; streptomycin, Himedia, code TC035 and Fetal bovine serum, FBS, code RM10679) was added along with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 mg/ml, 10  $\mu$ l) and incubate it for another 4h at carbon dioxide (5%) incubator. After incubation, formazon crystals were appeared and then dissolved in dimethyl sulfoxide (DMSO, Himedia TC185) solution. Optical density (OD) was measured at 570 nm using Elisa reader (Perkin Elmer).<sup>11,12</sup>

For measurement of TNF alpha production using PBMC cell culture supernatant collected from the treatment

of variable doses of aqueous root extract of *Ficus benghalensis* and *Ficus racemosa* along with NDV and/or IBD virus and this experiment was carried out in order to perform Sandwich Elisa and this experiment was performed according to the manufacturer's instructions, BD Biosciences.<sup>14</sup>

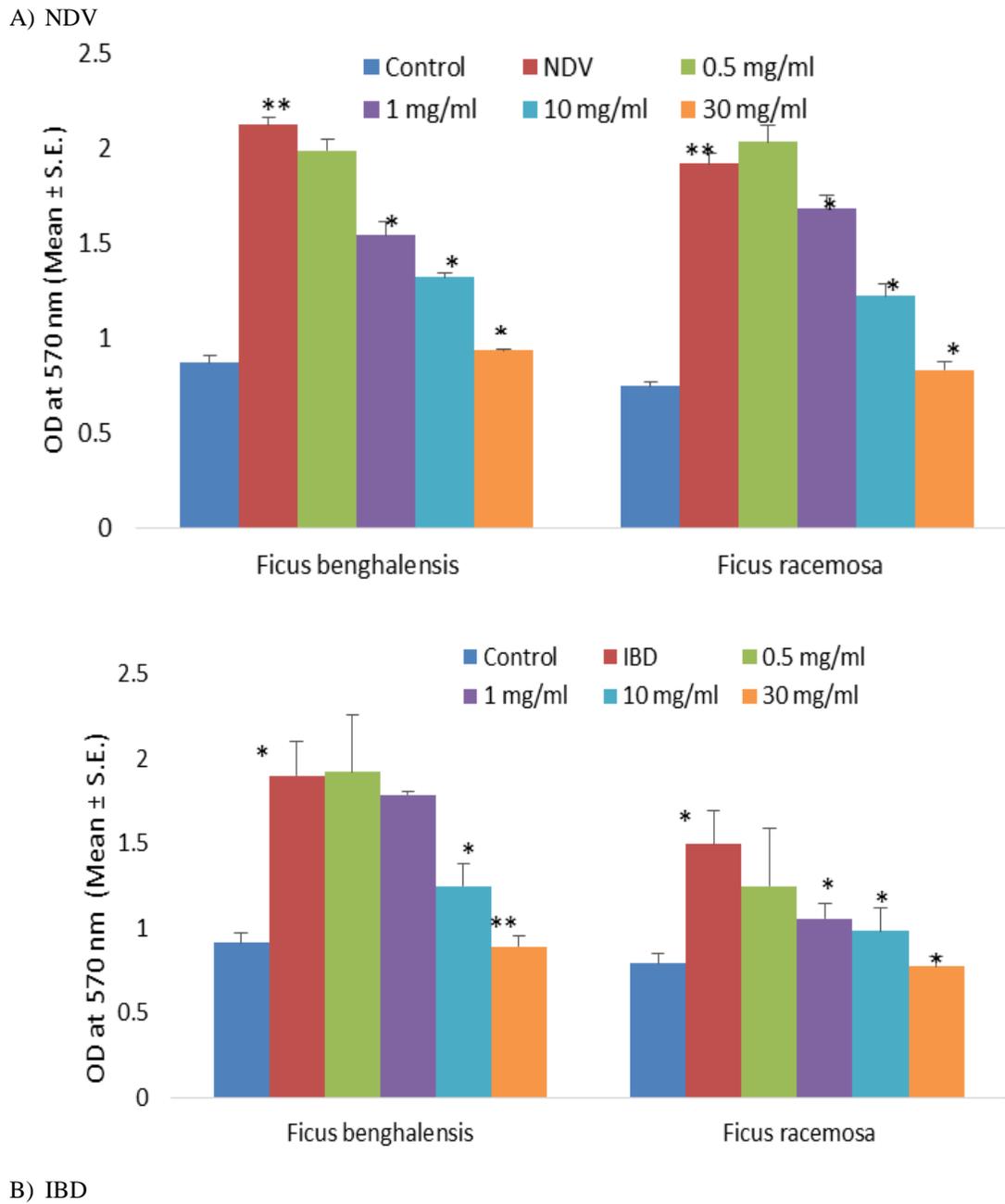
In this study, PBMC (settled at the bottom after centrifugation) samples of *Ficus benghalensis* and *Ficus racemosa* were stained with CD14 FITC (4  $\mu$ l) monoclonal antibody. The incubate samples at room temperature, lysed and washed with PBS (pH, 7.2). The resulting stained cell pellet was resuspended in 500  $\mu$ l of PBS and run on FACS Calibur (BD Biosciences), flow cytometer.<sup>2</sup>

All values were mentioned as Mean  $\pm$  S.E. Data was represented by one-way ANOVA test (Bonferroni multiple comparison test).

## RESULTS

Phytochemical investigation (qualitatively) of aqueous root extracts of *Ficus benghalensis* and *Ficus racemosa* revealed the presence of flavonoids and terpenoids. Alkaloids are totally absent in both the medicinal plants whereas saponin is present only in *Ficus benghalensis*.

The effect of aqueous root extract on PBMC proliferation assay (using NDV and IBD) has shown in Figure 1. The results showed that aqueous extract at higher doses showed significant inhibition in PBMC proliferation assay as compared to control. NDV and IBD used as standard and there is significant enhancement in proliferation as compared to control.

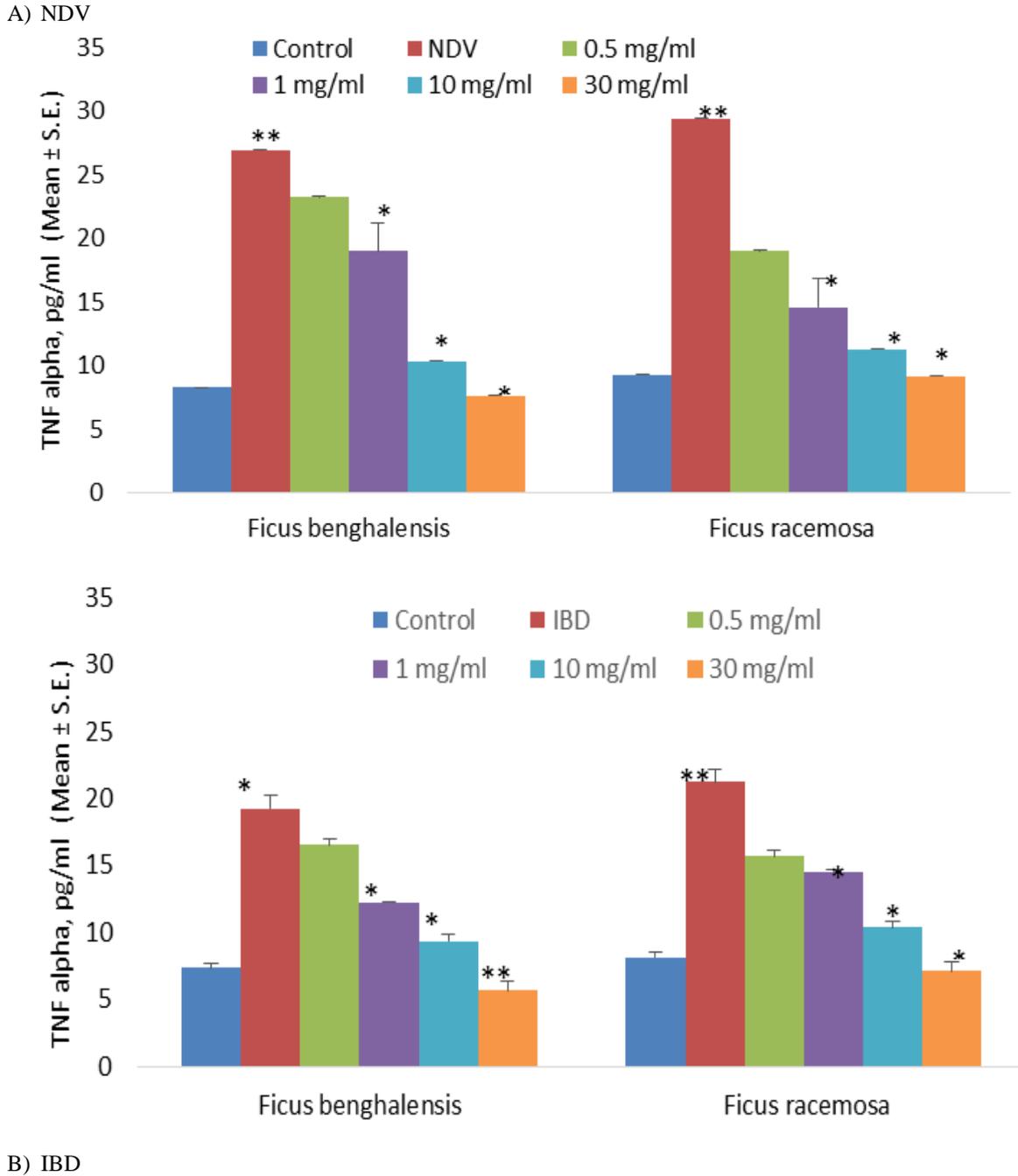


**Figure 1:** PBMC proliferation assay

Human PBMC ( $10^5$  cells/well) was cultured with NDV or IBD along with variable doses of aqueous root extract or NDV/IBD alone. After 48h, proliferation was measured by MTT assay. The results are presented as Mean  $\pm$  SE. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$  as compared to control.

As shown in Figure 2, the results showed that aqueous root extract at higher

doses significantly inhibited TNF alpha production as compared to control.

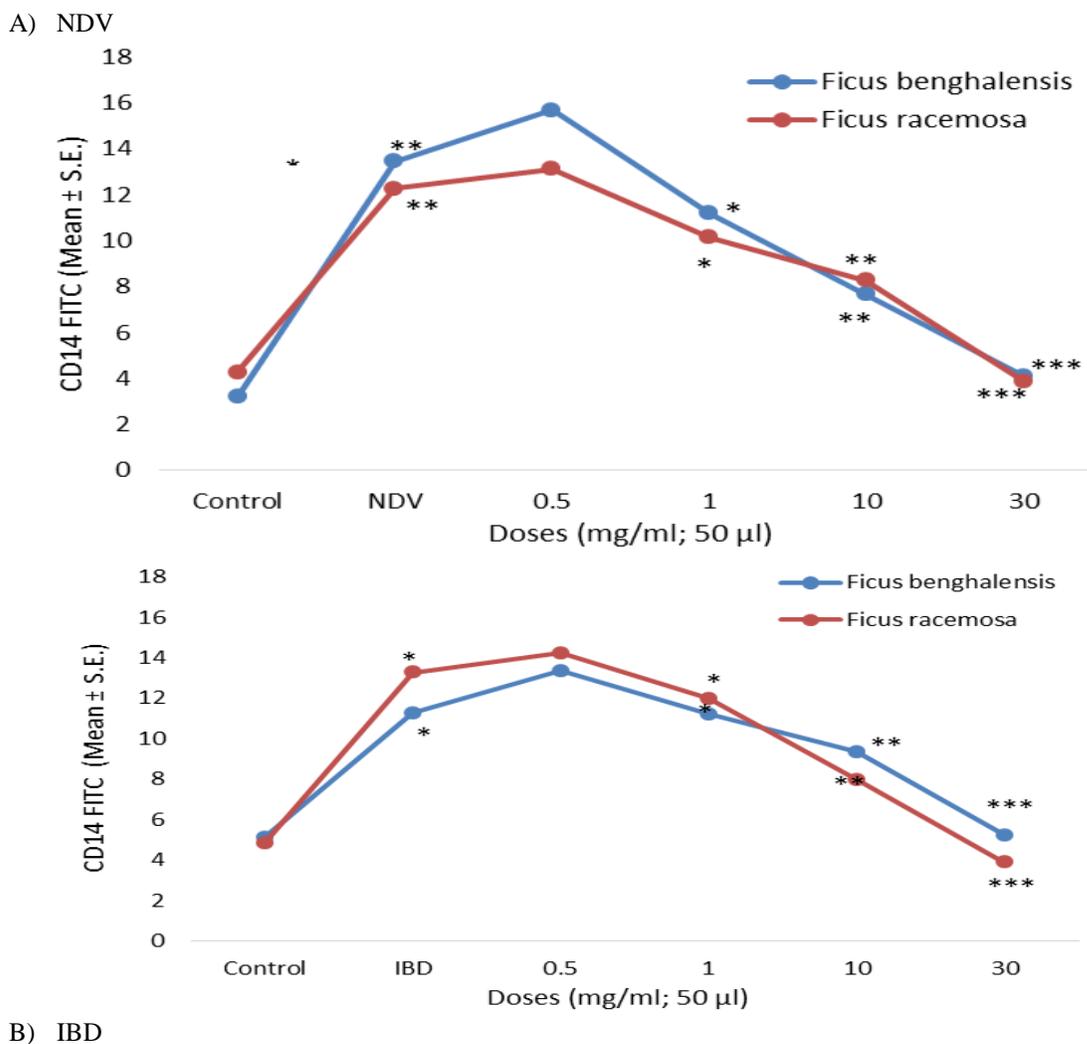


**Figure 2:** TNF alpha production from human PBMC culture supernatant

Human PBMC (105 cells/well) were cultured with NDV or IBD along with variable doses of aqueous root extract or NDV/IBD alone. After 48 h, supernatant was collected for the estimation of Th1 cytokine (TNF alpha). Values are expressed as Mean  $\pm$  S.E. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$  as compared to control.

The effect of aqueous root extract on CD14 monocyte surface marker using NDV and IBD has shown in Figure 3. At

higher doses, aqueous extract significantly inhibited CD14 monocyte surface marker as compared to control.



**Figure 3:** CD14 monocyte surface marker

Flow cytometric analysis of aqueous root extract (0.5-30 mg/ml; 50 µl) from *Ficus benghalensis* and *Ficus racemosa* to determine its effect (CD14, monocyte surface marker) in human PBMC against NDV and IBD. Data acquisition of 10000 events and fraction or separation of cell populations representing different phenotypes analyzed using cell quest software. Values are expressed as Mean ± SE. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

## DISCUSSION

The use of these medicinal plant products to heal or cure various infectious diseases has been extensively applied to the people worldwide. As per the literature survey and also our results supported and revealed the importance as well as potential of these medicinal plants for prophylactic and therapeutic treatment. In spite of this,

the number of medicinal plant products has not been completely investigated related to its phytochemical investigation and immunopharmacological based studies. In an effort to conduct these studies in order to search those compounds or metabolites that are present in the extract and showed antimicrobial activity,<sup>5,6,9</sup> qualitative based

studies need to be conducted to search for those metabolites that are responsible for its antimicrobial activity. The objective of our study was to invent safer antimicrobial agents from medicinal plant products and expected to be eco-friendly and easily obtainable.

The mechanisms of antimicrobial action of aqueous extract are not fully understood but several immunological studies have been conducted in this direction.<sup>7,8</sup> Although a number of research papers have been published related to identification of bio-active compounds in the aqueous extract, it is important to keep in mind that a single molecule that are present in aqueous extract may not be responsible for the observed activity but it may be responsible rather a combination of compounds interacting in an additive or synergistic manner.<sup>7,8</sup> For achieving this objective, group was identified and isolated viruses from suspected infected dead bird samples and determined its antimicrobial activity using aqueous root extract of *Ficus benghalensis* and *Ficus racemosa*. For antimicrobial studies, exact mechanism is almost clear between aqueous root extract of *Ficus benghalensis* and *Ficus racemosa* on human PBMC involving specific poultry viral antigen (NDV/IBD) activation. It showed identification and elucidation of the active constituent present in the aqueous extract of these 2 medicinal plants and may provide beneficial leads to the development of new and effective antimicrobial drugs. In this regard, preliminary results in the current study showed that aqueous root extract at higher doses showed inhibition in proliferation rate as compared to NDV/IBD control.

For antimicrobial studies especially Th1 (TNF alpha) cytokine production including CD14 monocyte surface marker provides general information about aqueous root extract of *Ficus benghalensis* and *Ficus racemosa* whether this aqueous root extract showed some inhibitory effects against these

two poultry diseases. To achieve this objective, poultry viruses (NDV and IBD) showed enhancement in CD14 surface marker, proliferation rate including TNF alpha production. The findings of these studies showed that the aqueous root extract of *Ficus benghalensis* and *Ficus racemosa* against NDV and IBD virus and the results are presented in respective figures and showed antimicrobial activity. As per the results, it gives clear indication about declining in TNF alpha production at higher doses of aqueous root extract against NDV/IBD. Generally, inhibitors of TNF alpha are generally given for the treatment of various inflammatory disorders. Over all the results indicate that aqueous root extract of *Ficus benghalensis* and *Ficus racemosa* shows a dosage-dependent correlation and found that aqueous root extract could significantly reduce the CD14 count in human PBMC exposed to NDV and IBD. The results of current immunopharmacological studies on human PBMC after exposing with NDV and IBD suggest that the aqueous leaves show antimicrobial effect on human PBMC.

## CONCLUSION

Immunopharmacological studies of *Ficus benghalensis* and *Ficus racemosa* are conducted on cell-mediated (T cell) immune response and at higher doses, aqueous extract significantly inhibited CD14 monocyte surface marker. Some of the medicinal plant products have shown antimicrobial activity. Further immunobiological research is mandatory to elucidate the most active constituents that are present in these aqueous extracts which may be useful or target molecule in the development of new effective and safe antiviral agents.

## CONFLICT OF INTEREST

Authors have declared that no conflicts of interest exist.

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