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Antimicrobial and Immunomodulatory Activities of Methanolic Extract of *Bauhinia vahlii*.

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ABSTRACT

Bauhinia vahlii is considered an important traditional folk medicine. In this study, methanol extract of *Bauhinia vahlii* and its various fractions were evaluated *in vitro* for their, antimicrobial as well as for their immunomodulatory properties on human peripheral blood mononuclear cells (PBMCs). The antibacterial efficacy was investigated by agar-well diffusion method and Minimum Inhibitory Concentration (MIC) against two Gram-positive and seven Gram-negative was determined. Furthermore, the immunomodulatory potential of the extracts was investigated through the MTT assay. The methanolic extract was found most effective (21 to 26mm) against *E. faecalis S. aureus, A. baumannii* and *C. freundii*. The MIC and MBC values of methanolic extract was found 1.51mg/mL and 3.41mg/mL against *E. faecalis* and *P. mirabilis* respectvily. The results of PBMCs Isolation and Cell Cultures showed that methanolic extract of *Bauhinia vahlii* significantly stimulated the proliferation of PBMCs *in vitro* in a dose-dependent manner.These findings indicate that methanolic extract of *Bauhinia vahlii* leaves showed high antibacterial activity and may be considered as an immunomodulatory agent.

Keywords: *Bauhinia vahlii,* antimicrobial, immunomodulatory.

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INTRODUCTION

Medicinal plants are the incredible and mostvaluable natural sources and have been used from centuries for the treatment of various diseases particularly for lesser side effect and better safety, efficacy and cultural acceptability [1]. Drug discovery from plant sources comprises a multidisciplinary approach such as ethnobotanical, phytochemical and biological techniques. Till date, they are continuing to provide us lead molecules for the development of drugs against various diseases. Recently, an attempt has been made to discover new antimicrobial agents from various kinds of microorganism, animal and plant sources[2].Today there is a urgent need for permanent search and development of new drugs from plant sources, as multi drug resistant bacterial strains are imposing vigorously. Similarly, the immune system is involved in pathophysiological mechanism of many diseases and enhancement of immune responses of the human beings to alleviate the diseases, which has now been the matter of discussion all over the world in the scietific community[3]. Immunomodulators are the substances that regulate the immune system by improving host defense mechanisms against diseases. The mechanism of immunomodulation is related to nonspecific activation of the function and efficiency of macrophages, granulocytes, natural killer cells and lymphocytes[4]. Nowadays, synthetic drugs used as mmunomodulatory agents are causing various infections and side effects throughout the immune system. Chemotherapeutic agents are reported to have mainly immunosuppressive activity and most of them are cytotoxic and cause various side effects. This information approaches a prime idea in search for investigating medicinal plants showing immunomodulatory activity[5].]. These problems can be overcome by boosting the immune system by the use of immunomodulatory drugs of natural origin as they are believed to enhance the natural resistance of the body against various infections[6].

There are many medicinal plants used in Indian Traditional System of Medicine are known to have immunomodulatory activities such as *Azadirachta indica, Terminalia chebula, Lawsonia alba, Ocimum sanctum, Boerhaavia diffussa, Withania somnifera, Asparagus racemosus, Nelumbo nucife, Calendula officinalis* etc[7]. The phytochemical compounds like terpenoids, steroids, proteins, flavonoids and tannins are considered to exhibit immunomodulatory activity. One of such resources in folk medicine is *Bauhinia vahlii* Wight & Arn (Fabaceae), which is the ever green tree and largest creeper and can grow up to 10-30m long. It is distributed in deciduous forests of India from Gujarat Southwards to Maharashtra and Northern Andhra Pradesh, commonly on hillsides and in forest valleys [8]. The leaves [11] very variable in size, often up to 18 inch. diameter, deeply cordate, 11-15 nerved,cleft through about 1/3 of the length, sub-coriaceous, dark green and the upper surface is more glabrescent and lobes are obtuse in shape. The leaves are cooked as vegetables. A decoction of the leaf is given to treat diarrhea and dysentery. Leaves are also used for fodder to make mats and for wrapping of tobacco for smoking[9]. It has been reported to contain amino acids, proteins[10].[11] It has also been reported to have betulinic acid, triterpene, campesterol and steroid in leaves[12].[Despite various therapeutic values of some species of *Bauhinia*, a prior investigation to validate the traditional applications have not been appeared in literature. The aim of this investigation was to evaluate the antimicrobial and immunomodulatory activities of the crude methanolic extract as well as various fractions of *B. vahlii* leaves.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Bauhinia vahlii* were collected randomly from the herbal garden of Regional Plant Resource Centre, Bhuabneswar, in the month of April, 2014. The authentification of this plant was confirmed by Dr. P. C. Panda, senior scientist, Regional Plant Resource Centre, Bhuabneswar, Odisha, India and the voucher specimen was preserved in Department of Pharmacognosy, Siksha 'O' Anusandhan University, Odisha, India.. Fresh leaves were washed under running tap water, air dried and then ground to coarse powder and stored in air tight container.

Preparation of extract

The air dried coarse powder (200 g) was extracted with methanol (2.5L) by maceration at room temperature for three days. The methanolic extract was filtered and concentrated to a dark viscous mass (Yield 17.6 % w/w) under reduced pressure at 50-55°C.The dried extract was suspended in distill water and was further fractionated by using different polarity based solents such as n-hexane, ethyl acetane and

chloroform successively. All these three fractions were collected and concentrated with vacuum rotary evaporator.The crude methanolic extract and these fractions obtained were investigated for immunomodulatory and antibacterial activity using standard methods.

Qualitative phytochemical analyses

Preliminary phytochemical screening of methanolic extract of *Bauhinia vahlii* was performed using standard method. (Harborne JB. Phytochemical methods. 3rd ed.London7 Chapman and Hall. 1984.)

Antibacterial activity assay

Bacterial strains

Gram-positive (*E. faecalis, S. aureus*) and Gram-negative (*A. baumannii, C. freundii, E. aerogenes, E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa*) bacteria were used as test organism and collected from Microbiology Department, Institute of Medical Sciences, Sum Hospital, Bhubaneswar, Odisha, India.

Antibacterial test of plant extract

One strain from each bacterial species having resistance to a maximum number of antibiotics was further used for monitoring antibacterial potentiality of methanolic extract, using the agar-well diffusion method, as detailed previously. Evaluation of antibacterial activities were done by measuring the diameter values of zones of inhibition after use of each extract and fraction. The experiment was conducted thrice and results are presented. It was confirmed that 10% DMSO had no inhibitory effect on any bacterium[13].

Determinations of MIC and MBC

Minimum inhibitory concentration (MIC) and *minimum bactericidal concentration* (MBC) of the all methanolic spice-extracts were determined. Original stock solutions of spice-extracts were prepared with methanol, at the concentration, 100 mg plant extract/mL in 10% DMSO solution with distilled water. Each stock solution was diluted suitably for final concentrations, 0, 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 mg/mL with the DMSO solution. Separate experiment was conducted for each spice-extract. An aliquot of 80 µl of each dilution of a extract was released to a well on a 96-welled (12 x 8) micro-titer plate, along with an aliquot of 100 µL MH broth (HiMedia), an aliquot of 20 µL bacterial inocula (10^9 CFU/mL) and a 5 µL-aliquot of 0.5 % of 2,3,5-triphenyl tetrazolium chloride (TTC). After pouring all the cited to a well, the micro-titre plate was incubated at 37°C for 18 h. The pink coloration development due to TTC in a well indicated bacterial growth and the absence of the colour was taken as the inhibition of growth. The first well of the micro-titre was the control without any extract. The MIC value was noted at the well, where no colour was manifested. Further, bacteria from each well of the micro-titre plate were sub-cultured on a nutrient agar plate; the dilution level, which caused no bacterial growth on the nutrient agar plate was observed, was taken as the MBC value[14].

PBMCs Isolation and Cell Cultures

Fresh blood was collected from healthy adult volunteers using heparinized tubes. Peripheral blood mononuclear cells (PBMCs) were separated by density-gradient centrifugation over Histopaque 1077(Sigma-Aldrich). The remnant erythrocytes in the recovered PBMCs layer were eliminated using lysis buffer to lysis the RBCs and washed three times in sterile phosphate buffered saline (PBS). The PBMCs were resuspended in 1 mL RPMI 1640 media (Sigma-Aldrich). The mononuclear cells were counted and adjusted to an appropriate concentration in complete RPMI 1640 supplemented with 2 mM glutamine, 1% penicillin-streptomycin antibiotic (Sigma-Aldrich) and containing 10% (v/v) fetal bovine serum (FBS) for further assays[15]. Their viability was determined by trypan blue exclusion test and only cell viability greater than 95%, as assessed by the trypan blue undergoes the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) MTT assay. The cells were then seeded on to 96-well flat bottom sterile tissue culture plates at a density of 5 × 104 cells/mL. All cell cultures were maintained at 37 °C.

% cell viability = [(absorbance of treatment group – blank)/ (absorbance of control group – blank)] X 100

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RESULTS

Preliminary phytochemical analysis

Preliminary phytochemical analysis of methanolic extract of *Bauhinia vahlii* leaves indicated the presence of carbohydrates, amino acids alkaloids, tannins, Glycosides, steroids and flavonoids.

Antibacterial efficacy of methanolic extract and fractions

The methanolic extract of *Bauhinia vahlii* leaves and its fractions were tested against antibacterial properties, by the agar-well diffusion method and values of zone of inhibition were recorded (Table 1). The methanolic extract was found most effective (21 to 26mm) against *E. faecalis S. aureus, A. baumannii* and *C. freundii*. The fractions such as n-hexane, chlorofom, ethyl acetate were clearly lesser in antimicrobial activity in comparison to former methanolic extract (Table 1). The methanolic leaf extract had registered the highest value of diameter size of zone of inhibition of 26 mm, against *S. aureus* and the lowest value of 19 mm against *E. aerogenes*. The n-hexane fraction showed the highest size of zone of inhibition of 25mm against *S. aureus* and lowest value of 19 mm against *P. mirabilis*, The chloroform extract was found highest value of diameter size of zone of inhibition 24 mm against *S. aureus* and the least size of zone of inhibition 12 against *P. mirabilis*. Similarly, the ethylacetate fraction had shown a highest value of 24 mm against *S. aureus* and failed control to *E. coli* and *A. baumannii.* Among all the extract and fractions, methanolic extract exhibited most effective anti bacterial activity.

Table 1: Antibacterial activity of *Bauhinia vahlii* by the agar well diffusion method.

Bacteria	Size of inhibition zones by extracts of twenty six spices (mm)			
	Methanol	n-hexane	Chloroform	Ethyl acetate
E. faecalis	25	24	21	22
S. aureus	26	25	22	24
A. baumannii	26	22	–	–
C. freundii	25	19	22	19
E. aerogenes	19	21	16	13
E. coli	21	26	–	–
K. pneumoniae	22	22	19	17
P. mirabilis	20	19	15	12
P. aeruginosa	26	23	12	15

MIC and MBC of methanolic extract and fractions

MIC and MBC values of methanolic extract and various fractions of *Bauhinia vahlii* were recorded (Table 2). MIC and MBC values of methanolic extract was found 1.51mg/mL and 3.41mg/mL against *E. faecalis* and *P. mirabilis* respectvily. Similarly, the MIC and MBC values of rest fractions were recorded (Table 2).

Table 2: MIC and MBC values of *Bauhinia vahlii* (mg/mL).

Strain	Methanol		n-hexane		Chloroform		Ethyl acetate	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis	1.51	3.41	1.51	3.41	3.41	4.27	3.41	4.27
S. aureus	1.51	3.41	1.51	3.41	3.41	4.27	1.51	3.41
A. baumannii	1.51	3.41	3.41	4.27	–	–	–	–
C. freundii	1.51	3.41	4.27	9.63	3.41	4.27	4.27	9.63
E. aerogenes	4.27	9.63	3.41	4.27			–	–
E. coli	3.41	4.27	1.51	3.41	–	–	–	–
K. pneumoniae	3.41	4.27	3.41	4.27	4.27	9.63	9.63	21.67
P. mirabilis	3.41	4.27	4.27	9.63	–	–	–	–
P. aeruginosa	1.51	3.41	4.27	9.63	–	–	–	–

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Immunomodulatory effect of methanolic extract

Methanolic extract of *Bauhinia vahlii* significantly stimulated the proliferation of PBMCs *in vitro* in a dose-dependent manner. In this experiment, after an incubation period of 24 h, methanolic extract at concentrations of 10, 20, 30, 40 µg/ml, registered cell viability of 10.56, 12.44, 16.23 and 19.83% respectively.(Fig. 1).

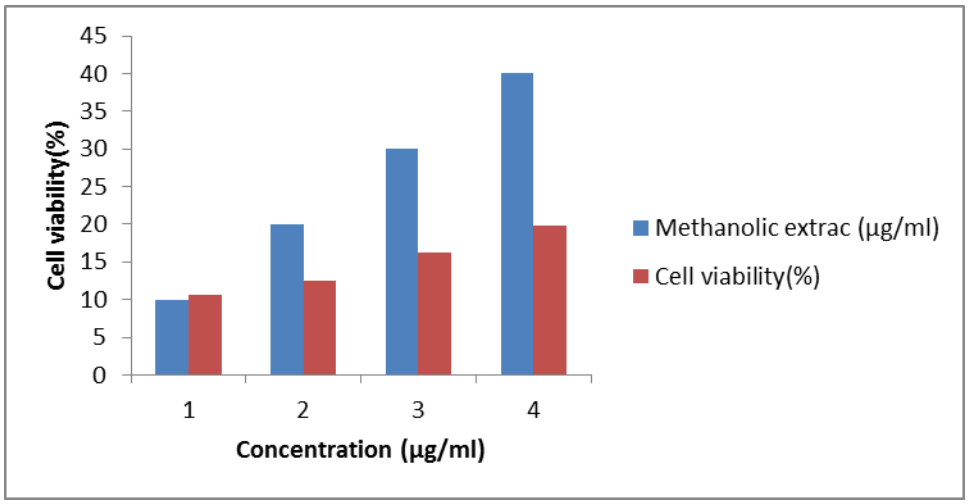

Figure 1: Percentage of PBMCs cell viability of *Bauhinia vahlii*

DISCUSSION

The Gram positive bacterial strains were more susceptible to the plant extracts as compared to gram negative bacteria. The results of antibacterial study of *Bauhinia vahlii* is in agreement with previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria[16]. It is widely reported that phenolic and flavonoid compounds may significantly contribute to overall antimicrobial activity[17].) The antibacterial activity of *Bauhinia vahlii* may be due to presence of bioactive compounds such as tannins, phenolic compounds, polyphenols and flavonoids[18].

Immunomodulatory agents of plant source increase the immune reponse of the body against various pathogens by activating the non-specific immune system[19].The immunostimulant agents have the ability to enhance the body's defense against various infections and cancer. The methanolic extract of *Bauhinia vahlii* leaves increases cell viability without causing toxicity on immune cells, which may be of value in combination with other therapies in the treatment of immunodeficiency, cancer, infections and autoimmune disorders. It also may be used as adjuncts to chemotherapy of cancer as it stimulated the PBMCs proliferation to more extent.The immunomodulatory activity of *Bauhinia vahlii* is also in agreement with the previous investigation[20].

Flavonoid glycosides and tannins are reported to have immunomodulatory activity [21]. As tannins and f lavonoid are found in methanolic extract of *Bauhinia vahlii*, our results support the immunomodulatory activity of this plant, and might be claimed as promoter of immune system against various disorders in the traditional system of medicine. It can be concluded that *Bauhinia vahlii* leaves possess antibacterial and immunomodulatory activity which may be due to the presence of flavonoids and tannins, and it could be useful for treating patients suffering from neutrophil function deficiency and bacterian infections.

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