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EVALUATION OF ANTI-INFLAMMATORY, ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF *PHYSORRHYNCHUS BRAHUICUS* HOOK LEAVES AND STEM EXTRACTS

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ABSTRACT

This study was planned to investigate the anti-inflammatory, antibacterial and antifungal activity of chloroform, ethyl acetate, hexane and methanol extracts of leaves and stem of *Physorrhynchus brahuicus* against sixteen bacterial and eight fungal isolates. The anti-inflammatory activity of extracts was studied by analyzing the inhibition of albumin denaturation. The antibacterial and antifungal activity of different concentrations of extracts was analyzed by performing well-plate method. The methanol extract of leaves and stem showed maximum inhibition of albumin denaturation while chloroform extract of stem also showed inhibition but not considerable. In case of antifungal activity of leaves; the methanol, hexane and ethyl acetate extracts showed significant zones of inhibition compared with the zones of antibiotic nystatin. On the other hand; the hexane, chloroform and methanol extracts of stem showed considerable larger zones as compared to nystatin. These results justify that the inhibition by these extracts were similar and even more at various concentrations as the antibiotic nystatin showed. The largest zones of inhibition were observed at 5000ug concentration of approximately all the used leaves and stem extracts. In case of antibacterial activity, non-significant zones of inhibition were observed in all the extracts treatment. All the extracts of leaves and stem showed potential antifungal but very less antibacterial activity. These findings may support the use of *Physorrhynchus brahuicus* as an anti-inflammatory and antifungal traditional plant.

Keywords:*Physorrhynchus brahuicus*, leaves and stem extracts, anti-inflammatory, antibacterial, antifungal, albumin denaturation

INTRODUCTION

Plants contain many compounds having an antimicrobial activity and they are used in the treatment of various infections (Chaddha *et al.*, 2015). *Physorrhynchus brahuicus* Hook is a plant belongs to the family Brassicaceae. It is distributed in various areas of Pakistan, India and Afghanistan. In Pakistan, it is found in Hala range (Sindh), in salt range (Punjab), in Sibbi and Bugti hills (Balochistan). The plants of the aforementioned family are used for edible purposes, preparation of medicines, ornamental purposes and have oil producing seeds.

Inflammation is the process associated with many infectious as well as non-infectious diseases. Many drugs have been designed to treat several abnormal conditions including infectious diseases, cancer, autoimmune disorders, cardiovascular and bowel diseases, diabetes mellitus and arthritis. However, there is still need to develop new drugs to tackle with disease associated inflammatory processes (Hunter, 2012; Chen *et al*., 2018). Tissue damage initiates the inflammation leads to the release of different chemical mediators and chemotactic factors. It is reported that many phytochemicals have been proved as anti-inflammatory agents (Govindappa *et al*., 2011).

Bacteria cause a variety of diseases in humans, animals as well as in plants. For example; *Klebsiella* has an ability to cause pneumonia, bacteremia, meningitis and urinary tract infections in humans (Kumar *et al.*, 2016; Jayaraj *et al*., 2014). *Proteus marbilis* is ubiquitous and can cause empyema and osteomyelitis (Bahashwan and Shafay, 2013) and *Shigella dysenteriae* cause serious infections because of the production of shiga toxin (Omololu-Aso *et al.*, 2017). *Staphylococcus aureus* is an important human pathogen that is known to cause minor skin infections to serious diseases (Huang *et al.*, 2009). It is reported that about 250,000 people died per year globally because of the typhoid fever which is caused by *Salmonella typhi* (Saleh *et al.*, 2014). *Salmonella typhi para A* cause enteric fever usually known as paratyphoid (Naveed and Ahmed, 2016). *Pseudomonas aeruginosa* is responsible for causing pneumonia and also severely affects the lungs of patients suffering from cystic fibrosis (Debarbieux *et al.*, 2010). *Escherichia coli* is associated with urinary tract infections mainly cystitis (Kariuki *et al.*, 2007) and *Enterococcus faecalis* is now ranked among the top three nosocomial bacterial pathogens (Kayaoglu and Orstavik, 2004). *Staphylococcus saprophyticus* is a causative agent of urinary tract infections (UTIs) in young and adult females (Raz *et al.*, 2005; Widerstrom *et al.*, 2012). *Staphylococcus epidermidis* can cause conjunctivitis and

endophthalmitis (Dave *et al.,* 2011). Methicillin resistant *Staphylococcus aureus (MRSA)* cause infections associated with high mortality rate including nasal infections (Parasa *et al.,* 2011; Chao *et al.,* 2008). *Bacillus species* are generally associated with food poisoning, sepsis, meningitis, endocarditis, endophthalmitis and respiratory diseases (Ozkocaman *et al.,* 2006).

Fungi are also the major cause of human diseases. *Aspergillus flavus, Aspergillus niger, Candida* species, *Mucor* species, *Saccharomyces cerevisae, Penicillium species, Trichophyton mentagrophytes* and *Microsporum gypseum* are the most important human pathogens cause serious infections. *Trichophyton mentagrophytes* and *Microsporum gypseum* are the well known dermatophytes and cause the infections of nails, hairs and skin.

MATERIALS AND METHODS

In the present study, the anti-inflammatory, antifungal and antibacterial effect of stem and leaves extracts of *Physorrhynchus brahuicus* was observed. The fungi included *Aspergillus flavus, Aspergillus niger, Candida* species, *Mucor* species, *Saccharomyces cerevisae, Penicillium* species, *Trichophyton mentagrophytes* and *Microsporum gypseum* was investigated. Moreover, effect was also observed on different bacterial isolates including *Klebsiella pneumoniae, Proteus marbilis, Shigella dysenteriae, Staphylococcus aureus, Salmonella typhi, Salmonella typhi para A, Salmonella typhi para B, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Staphylococcus saprophyticus, Staphylococcus epidermidis,* Methicillin resistant *Staphylococcus aureus (MRSA), Bacillus subtilis, Micrococcus luteus* and *Corynebacterium xerosis.* Different concentrations of each extract was prepared in DMSO i.e. 250µg, 500µg, 750µg, 1000µg, 5000µg for antibacterial activity and 250µg, 750µg, 1500µg, 3000µg, 4000µg and 5000µg for the antifungal activity. DMSO was used as a negative control. Nystatin and other antibacterial antibiotics were used as positive control to compared the activity of various extracts (Nostro *et al.,* 2000).

Collection of Herbs

The stem and leaves parts of *Physorrhynchus brahuicus* were collected from Sehwan Sharif, Sindh (G.H no. 93758). After collection, it was identified by the taxonomist in Herbarium, Center for Plant Conservation situated in the University of Karachi, Karachi.

Preparation of Herbal Extracts

For the preparation of herbal extracts, the stem and leaves of *Physorrhynchus brahuicus* were washed, dried, crushed and ground to convert into powdered form. The Soxhlet apparatus was used to make different extracts of the collected plant. Fifteen grams of powdered plant material was weighed and wrapped in whatsman 41 filter paper. The plant material was placed inside the extraction tube, over which a condenser was fixed. The soxhlet apparatus was connected to a distillation flask and a chiller which was set at temperature 5˚C. 150 ml of respective solvent i.e. hexane, chloroform, ethyl acetate and methanol was poured in the distillation flask placed on a heating mantle. The temperature was adjusted in accordance with the solvent used (30˚C-40˚C). The extraction process was continued for about 14 hours. The extract was then transferred to a round bottom flask to concentrate using BÜCHI Rota-vapour R-200. The flask containing the extract sample was submerged in a water bath set to the temperature 40˚C. The concentrated extract was partitioned in the extract tube and left opened for the removal of any residual solvent. The dried form of extract was kept at 4˚C for experimental procedures.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

The reaction mixture was comprised of test extracts and 1% aqueous solution of bovine albumin fraction. Extracts were used in 200ug concentration as aspirin was used. All the samples were incubated at 37^0C for 20 minutes and then heated at 51^0C for 20 minutes. After cooling, the turbidity of the samples was measured using spectrophotometer at 660nm. Percent inhibition of protein denaturation was calculated (Govindappa *et al*., 2011) by applying the formula i.e. % inhibition= [{Abs control- Abs sample}/Abs control] x 100,
(Abs control = the absorbance without sample, Abs sample = the absorbance of sample extract/standard)

Effect of plant extracts on bacterial and fungal isolates

For the preparation of plant extracts, different solvents were used including methanol, ethyl acetate, hexane and chloroform. DMSO was used to make different concentrations of four types of extracts i.e. 250µg (5%), 500µg (10%), 750µg (15%), 1000µg (20%), 5000µg (100%) for antibacterial activity and, 250µg (5%), 750µg (15%),

1500µg (30%), 3000µg (60%), 4000µg (80%) and 5000µg (100%) for antifungal activity. Extract concentrations were selected after performing MIC assay.

Preparation of lawns

Mueller Hinton and Sabouraud's dextrose agar were used to see the antibacterial and antifungal activities respectively by performing well-diffusion technique. 0.5 McFarland's index was prepared to get the inoculum size of 1.5 x 10^8CFU/ml (Coyle, 2005). 0.1ml was added and lawns were prepared by the help of spreader. After certain period of time, wells were made on each plate with the help of a borer. 50µl of different concentrations of extracts were transferred to the wells and incubated for 24 hours (Nostro *et al.*, 2000).

Measurements of zone of inhibition

After incubation, the plates were observed for the zone of inhibition around the wells and the diameter was measured around each well in millimetres (mm).

Statistical Analysis

Statistical analysis was performed by using the software IBM SPSS Statistics 23. One way analysis of variance (ANOVA) followed by Bonferroni post hoc test and student's t-test were performed to compared the groups with level of confidence $P < 0.05$; (where * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Data are presented as mean ± SEM.

RESULTS

Anti inflammatory activity

Inhibition of albumin denaturation

A major cause of inflammation is the denaturation of proteins. We investigated the effect of different extracts of leaves and stem of *Physorrhynchus brahuicus* on protein denaturation. Our results showed that few of our extracts were effective in inhibiting heat induced albumin denaturation (Table 1). Maximum inhibition was observed from methanol extract of leaves and stem i.e. 69.32 and 61.67, respectively. Chloroform extract of stem also showed inhibition i.e. 40.21. On contrary, other extracts did not show good inhibition. Aspirin was used as a standard anti-inflammatory drug (Govindappa *et al*., 2011) and showed the maximum inhibition i.e. 75.22 at the concentration of 200 µg.

Effect of stem extracts on fungal isolates

Chloroform extract

The zone of inhibition against *A.niger* was observed at 250µg, 750µg, 1500µg, 3000µg, 4000µg and at 5000µg, larger zone was observed which was greater than nystatin. No zone of inhibition was observed in case of *M.gypseum* and *T.mentagrophytes* when treated with all the concentrations of extract. *Penicillium* species showed sensitivity to all the concentrations of extract. The zone of inhibition against *Mucor* species was observed at 750µg, 1500µg and 3000µg ($p < 0.01$**). At 250µg, 4000µg and 5000µg, the zones of inhibition were statistically non-significant as compared to nystatin. In case of *S.cerevisae*, zones were appeared at all the concentrations of extract ($p < 0.01$**). The zones of inhibition against *A.flavus* were statistically non-significant as compared to nystatin at all the concentrations of extract. In case of *Candida* species, at 250µg, 3000µg and 5000µg, the diameter of zones were close to the diameter of zone of nystatin. At 4000µg, larger zone of inhibition was observed as compared to nystatin. At 750µg and 1500µg, zones were also observed (Table 2a).

Ethyl Acetate extract

The zone of inhibition against *A.flavus* was smaller than nystatin at all the concentrations of extract ($p < 0.001$***). No zone of inhibition was observed in case of *M.gypseum* when treated with all the concentrations of extract. In case of *Mucor* species, zones of inhibition were statistically non-significant and approximately same as compared to the zone of nystatin against *Mucor*. The zones of inhibition against *S.cerevisae* were smaller at all the concentrations of the extract. In case of *T.mentagrophytes*, non-significant zones were appeared at all the concentrations of the extract except, 5000µg, on which zone of inhibition was close to the zone of nystatin. The zones of inhibition against *A.niger* were statistically non-significant at all the concentrations of the extract as compared to nystatin. In case of *Candida* and *Penicillium* species, statistically non-significant zones were observed at all the concentrations of extract as compared to nystatin. At 5000ug, larger zone was observed in case of *Candida*, close to the diameter of nystatin (Table 2b).

Table 1. Effect of different solvent extracts of *Physorrhynchus brahuicus* on albumin denaturation.

Extracts	Leaves (O.D)	Stem (O.D)
Methanol (200µg/mL)	69.32±0.06	61.67±0.09
Chloroform (200µg/mL)	30.22±0.07	40.21±0.05
Hexane (200µg/mL)	32.21±0.08	30.98±0.02
Ethyl acetate (200µg/mL)	29.00±0.06	25.45±0.07
Aspirin (200µg/mL) 75.22±0.09 (standard)		

Table 2a. Antifungal activity of Chloroform extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	Zone of inhibition at different concentrations (mm) STEM						Zone of inhibition at different concentrations (mm) LEAVES						Zone of inhibition (mm)
	5%	15%	30%	60%	80%	100%	5%	15%	30%	60%	80%	100%	Nystatin
A.flavus	2 ±0.5	2.33 ±0.6	3.33 ±1.2	3.33 ±1.6	2.66 ±1.3	2.66 ±0.8	1 ±00	1 ±00	1 ±00	1 ±00	1.66 ±0.3	2 ±00	4±0.1
A.niger	4 ±0.5	2.33 ±0.3	1.66 ±0.3	2.33 ±0.6	3 ±0.5	6 ±0.5	0.66 ±0.3	1 ±00	1 ±00	1.33 ±0.3	1.66 ±0.3	2 ±0.5	5±0.4
Candida	3.33 ±0.3	2.66 ±0.8	2 ±1	3.33 ±0.6	6 ±0.5	4.33 ±0.6	1 ±00	1 ±00	2 ±1	4.33 ±0.3	5 ±0	4 ±0.5	4.5±0.4
M.gypseum	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±0	0 ±0	6±0.2
Mucor	3.33 ±0.8	2 ±1	1.66 ±0.6	1.33 ±0.3	5.66 ±0.8	6 ±0.5	2.33 ±0.6	2.66 ±0.8	3 ±0.5	3 ±0.5	3.33 ±0.3	5.33 ±0.6	3.5±0.5
Penicillium	2 ±00	2 ±0.5	2 ±00	1.66 ±0.3	2.33 ±0.6	2.66 ±0.3	2.66 ±0.3	2.66 ±0.3	3.66 ±0.3	3 ±0.5	2.66 ±0.6	3.33 ±0.6	4.5±0.1
Saccharomyces	2.66 ±0.3	2.66 ±0.3	2 ±0.5	1.66 ±0.6	3 ±0.5	3.33 ±0.3	1 ±00	1 ±00	1 ±00	1.33 ±0.3	2 ±0	2.33 ±0.6	4±0.8
T.mentagrophytes	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	5.33 ±1.7	2 ±1.5	3.33 ±2.8	2.33 ±0.3	2 ±0	5.33 ±2.7	3±0.6

Hexane extract

No zone of inhibition was observed in case of *M.gypseum* and *Penicillium* species when treated with all the concentrations of extract. In case of *Mucor* species, large zone of inhibition was produced as by nystatin. At 1500µg and 4000µg concentrations, zones were close to the diameter of nystatin zone. The zones of inhibition against *S.cerevisae* were observed at all the concentrations of the extract. The zones of inhibition against *T.mentagrophytes* were close to the zone of nystatin at all the concentrations of extract. *A.flavus* showed increase in the zone of inhibition with the increase in the concentration of extract. At concentrations 250µg and 750µg, zones were close and same to the zone of nystatin. At 1500µg, 3000µg, 4000µg and 5000µg, the zones of inhibition were larger as compared to the zone of inhibition produced by nystatin. In case of *A.niger*, small zones of inhibition were appeared as compared to the zone of nystatin. The zones of inhibition against *Candida* species were close to the zone of nystatin at all the concentrations of extract (Table 2c).

Methanol extract

In case of *A.flavus*, non-significant zones were observed. The zones of inhibition against *A.niger* were small at all the concentrations as compared to nystatin. No zone of inhibition was observed against *M.gypseum* and *T.mentagrophytes* at any concentration of extract. *Penicillium* species, *Candida* species and *S.cerevisae* showed smaller zones of inhibition. In case of *Mucor* species, the zones of inhibition at 250µg, 4000µg and 5000µg were comparatively larger as compared to nystatin. At concentration 750µg, 1500µg and 3000µg, considerable zones were measured (Table 2d).

Effect of leaves extracts on fungal isolates

Chloroform extract

When the fungal isolates were treated with chloroform extract of leaves, it was observed that in case of *A.flavus*, *A.niger* and *S.cerevisae*, small zones of inhibition were observed at all the concentrations of extract ($p < 0.001$***). In case of *Candida* species, at 3000µg and 5000µg considerable zones of inhibition were observed. While at 4000µg,

larger zone was observed as compared to the zone of nystatin against *Candida*. The zones of inhibition against *Mucor* species were good at 250µg, 750µg, 1500µg, 3000µg and 4000µg. At 5000µg, larger zone of inhibition was found as compared to nystatin. In case of *Penicillium* species, considerable zones were obtained at all the concentrations of extract. Statistically non-significant zones were obtained in case of *T.mentagrophytes* on comparison with nystatin. At concentrations 250µg and 5000µg, very large zones of inhibition were produced as compared to nystatin. No zone of inhibition was observed against *M.gypseum* at all the concentrations used (Table 2a).

Ethyl Acetate extract

At all the concentrations of extract, small zones were observed ($p<0.001$***) against *Candida* species and *S.cerevisae* as compared to nystatin. In case of *M.gypseum*, *T.mentagrophytes* and *Penicillium*, no zone of inhibition was observed at all the concentrations. In case of *A.flavus*, at 250µg, 750µg, 1500µg and 3000µg, observable zones were produced. At 4000µg and 5000µg, considerably larger zones were observed as compared to nystain. Statistically non-significant zones of inhibition were observed in case of *A.niger* at all the concentrations of extract. *Mucor* species was sensitive to all the concentrations of extract and showed larger zones on comparison with nystatin (Table 2b).

Table 2b. Antifungal activity of Ethyl acetate extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	Zone of inhibition at different concentrations (mm) STEM						Zone of inhibition at different concentrations (mm) LEAVES						Zone of inhibition (mm)
	5%	15%	30%	60%	80%	100%	5%	15%	30%	60%	80%	100%	Nystatin
A.flavus	1.33 ±0.3	1.33 ±0.3	0.66 ±0.3	0.33 ±0.3	1.33 ±0.3	1 ±00	3.66 ±0.6	4 ±1	4.66 ±1.3	4.66 ±1.3	5.66 ±0.8	7 ±00	4±0.1
A.niger	4 ±0.5	4 ±1	2.33 ±0.3	2.33 ±0.3	4 ±1	3 ±0.5	2 ±1.1	2.66 ±1.3	2.66 ±1.3	3.66 ±2	3.33 ±1.7	3 ±1.5	5±0.4
Candida	3.66 ±0.6	2.66 ±0.3	2.33 ±0.3	3.33 ±0.6	3.33 ±0.8	4 ±0.5	1 ±00	1 ±00	1 ±00	1 ±00	1 ±00	1 ±00	4.5±0.4
M.gypseum	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	6±0.2
Mucor	3.33 ±0.6	3.66 ±0.3	3.66 ±0.3	3.66 ±0.6	3 ±00	3 ±0.5	4.66 ±0.6	4.33 ±0.8	5 ±0.5	4.66 ±1.2	5 ±1	6 ±0.5	3.5±0.5
Penicillium	3.66 ±0.1	3.33 ±00	3.66 ±00	3 ±0.1	3.33 ±00	3 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	4.5±0.1
Saccharomyces	1 ±00	1.66 ±0.6	1.66 ±0.6	1.66 ±0.6	1 ±00	1 ±00	1 ±00	1 ±00	1 ±00	1 ±00	1 ±00	1 ±00	4±0.8
T.mentagrophytes	1 ±00	0.33 ±0.3	0.33 ±0.3	0.33 ±0.3	1 ±0.5	2.66 ±0.3	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	3±0.6

Table 2c. Antifungal activity of Hexane extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	Zone of inhibition at different concentrations (mm) STEM						Zone of inhibition at different concentrations(mm) LEAVES						Zone of inhibition (mm)
	5%	15%	30%	60%	80%	100%	5%	15%	30%	60%	80%	100%	Nystatin
A.flavus	3.66 ±0.6	4 ±1	4.66 ±1.3	4.66 ±1.3	5.66 ±0.8	7 ±00	3.3 ±0.6	3.33 ±0.3	3.3 ±0.3	2.33 ±0.3	2.66 ±0.3	3.66 ±0.3	4±0.1
A.niger	2 ±1.1	2.66 ±1.3	2.66 ±1.3	3.66 ±2	3.33 ±1.7	3 ±1.5	1.6 ±0.3	1.65 ±0.3	1.66 ±0.3	1.56 ±0.3	1.5 ±0.3	2.66 ±0.3	5±0.4
Candida	4 ±0.5	3.66 ±0.8	4 ±00	4.33 ±0.8	4.33 ±0.8	4.33 ±0.6	2.33 ±0.3	2.4 ±0.3	3.33 ±0.3	3.60 ±0.3	4 ±00	4 ±0.5	4.5±0.4
M.gypseum	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	6±0.2
Mucor	4.66 ±0.6	4.3 ±0.8	5 ±0.5	4.66 ±1.2	5 ±1	6 ±0.57	3 ±00	3 ±0.5	3.66 ±0.3	4 ±00	3.66 ±0.3	5 ±00	3.5±0.5
Penicillium	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	4.5±0.1
Saccharomyces	2.33 ±0.8	2 ±1.1	2.33 ±0.6	2.66 ±0.3	2 ±0.5	2.33 ±0.8	2 ±00	3 ±00	3 ±00	3.33 ±0.3	4 ±00	4.66 ±0.3	4±0.8
T.mentagrophytes	2.66 ±0.6	2.66 ±0.3	2.66 ±0.6	2 ±00	3±00	3.33 ±0.6	4 ±1.5	5 ±1	5 ±1.5	3.66 ±0.3	3 ±0.5	5.66 ±1.2	3±0.6

Table 2d. Antifungal activity of Methanol extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	Zone of inhibition at different concentrations (mm) STEM						Zone of inhibition at different concentrations (mm) LEAVES						Zone of inhibition (mm)
	5%	15%	30%	60%	80%	100%	5%	15%	30%	60%	80%	100%	Nystatin
A.flavus	1 ±0.5	1 ±00	1 ±00	1.33 ±0.3	1.33 ±0.3	2.33 ±0.3	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	4±0.1
A.niger	2.66 ±0.3	2.66 ±0.3	3 ±00	2.66 ±0.3	3 ±0.5	3.66 ±0.3	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	5±0.4
Candida	2.33 ±0.6	1.33 ±0.6	1 ±0.5	1 ±1	1.66 ±0.8	3.33 ±0.8	1.66 ±1.2	0.66 ±0.3	0.66 ±0.3	0.66 ±0.3	1.33 ±0.6	3.33 ±1.7	4.5±0.4
M.gypseum	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	6±0.2
Mucor	6.33 ±2	4.66 ±1.2	2.66 ±1.4	5.33 ±1.4	7 ±2.6	7.66 ±2.3	0.66 ±0.3	1.33 ±0.3	1.33 ±0.3	1 ±00	1 ±00	0.66 ±0.3	3.5±0.5
Penicillium	1 ±00	1 ±00	1 ±00	1 ±00	1 ±00	2 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	4.5±0.1
Saccharomyces	1 ±00	0.66 ±0.3	1 ±0.5	0.66 ±0.3	1.33 ±0.8	1.66 ±0.3	1.33 ±0.3	1 ±00	1 ±00	1 ±00	1.33 ±0.3	2 ±0.5	4±0.8
T.mentagrophytes	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	7.33 ±0.8	4 ±2	4.66 ±0.6	4 ±1.5	2 ±0.5	7.33 ±1.2	3±0.6

Table 3a. Antibacterial activity of Chloroform extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	Zone of inhibition at different concentrations (mm) STEM					Zone of inhibition at different concentrations (mm) LEAVES					Zone of inhibition (mm)
	5%	10%	15%	20%	100%	5%	10%	15%	20%	100%	
Proteus mirabilis	1.5 ±0.6	0 ±00	0.8 ±0.4	0.8 ±0.4	1.33 ±0.33	1.66 ±0.4	1 ±0.5	1 ±0.5	0.83 ±0.4	1.33 ±0.7	Van=8±0.3
Micrococcus luteus	0.9 ±0.2	1 ±00	1 ±00	0.6 ±00	1.16 ±0	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Amp= 10±0.4
Shigelladysentriae	0 ±00	0 ±00	0 ±00	0 ±00	1 ±0.16	1 ±00	1 ±00	1 ±00	1 ±00	1 ±0.16	Gen= 19±0.3
Salmonella typhi	0.5 ±0.5	0.3 ±0.3	1.16 ±0.16	1.5 ±0.28	2.3 ±0.16	0.6 ±0.3	0.6 ±0.3	0 ±00	0.3 ±0.2	1.5 ±0.2	Van = 6±0.6
Salmonella typhipara A	0.66 ±0.3	0.66 ±0.3	0.66 ±0.3	0.66 ±0.3	1 ±00	0.6 ±0.3	1 ±0.5	1 ±0.5	1 ±0.5	1.66 ±0.8	Strep=13±0.7
Salmonella typhipara B	0 ±00	0 ±00	0.66 ±0.3	0 ±00	1 ±00	1.5 ±00	1.33 ±0.16	0.3 ±0.2	0.3 ±0.2	1 ±00	Strep=13±0.9
Staphylococcus aureus	1.5 ±0.5	1 ±00	0.66 ±0.33	1 ±00	1.16 ±0.16	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	0.66 ±0.3	1.66 ±0.8	Pen = 4±0.4
MRSA	1 ±0.5	0.83 ±0.4	0.66 ±0.3	1.16 ±0.6	1.66 ±0.8	1 ±00	0.66 ±0.33	1.16 ±0.16	1.66 ±0.44	2.33 ±0.3	Met = 6±0.32
Streptococcus fecalis	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	0.66 ±0.3	Nov = 17±00
Pseudomonas aeruginosa	1 ±00	1±0	1 ±00	1 ±00	1.16 ±0.16	0 ±00	0.5 ±0.2	1 ±00	1 ±00	2.8 ±0.4	Van = 5±0.1
Escherichia coli	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	2.1 ±0.6	Van = 8±0.12
Klebsiella pneumonia	1.33 ±0.33	1.33 ±0.33	1.33 ±0.16	1 ±00	1.66 ±0.33	0.66 ±0.33	0.66 ±0.33	0.3 ±0.2	0.3 ±0.2	1 ±00	Amp= 12±0.5
Corynebacteriumxerosis	0.5 ±0.25	0.33 ±0.25	0.66 ±00	0.33 ±0.25	1 ±00	0 ±00	0 ±00	0.3 ±0.2	0.3 ±0.2	1 ±00	Poly= 16±0.3
Staphylococcus epidermidis	1.66 ±0.3	1.66 ±0.3	2 ±0.28	2 ±0.44	2 ±0.28	0.3 ±0.2	0.3 ±0.2	0.6 ±0.33	0.6 ±0.33	0.6 ±0.3	Rif = 10±0.3
Staphylococcus saprophyticus	0.33 ±0.2	0.33 ±0.25	0 ±00	0 ±00	1 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Eryth=14±0.3
Bacillus subtilis	0.33 ±0.33	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	1.66 ±0.16	1.33 ±0.33	1.5 ±0.5	1.5 ±0.28	1.83 ±0.6	2.33 ±0.6	Nov = 16±00

Van=Vancomycin, Amp=Ampicillin, Gen=Gentamycin, Strep=Streptomycin, Pen=Penicillin, Met=Methicillin, Nov=Novobiocin, Poly=Polymixin, Rif=Rifampin, Eryth=Erythromycin

Hexane extract

A.niger showed sensitivity to all the concentrations of extract but small zones of inhibition found at all the concentrations of extract. In case of *Mucor* species, at 250µg and 750µg, observable zones were produced ($p < 0.001$***). At 1500µg, 3000µg, 4000µg and 5000µg, larger zones of inhibition were observed as compared to the zone produced by nystatin. The zones of inhibition against *S.cerevisae* and *Candida* species were significant at all the concentrations of extract. Zones of inhibition increased with the increase in concentration of extracts. In case of *T.mentagrophytes*, statistically non-significant and larger zones of inhibition were observed at all the concentrations of extract when compared to the zone of nystatin. In case of *A.flavus*, observable zones were observed at all the concentrations of extract. No zone of inhibition was observed at any concentration of extract in case of *M.gypseum* and *Penicillium* (Table 2c).

Table 3b. Antibacterial activity of Hexane extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	Zone of inhibition at different concentrations (mm) STEM					Zone of inhibition at different concentrations (mm) LEAVES					Zone of inhibition (mm)
	5%	10%	15%	20%	100%	5%	10%	15%	20%	100%	
Proteus mirabilis	1 ±0.5	3.33 ±1.4	3.33 ±1.2	0.66 ±0.66	4.66 ±0.88	0 ±00	0 ±00	0 ±00	0 ±00	1 ±0.33	Van=8±0.3
Micrococcus luteus	1 ±0.1	1 ±0.16	1 ±0.16	1 ±0.16	1 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Amp= 0±0.4
Shigelladysentriae	1 ±00	0.33 ±0.2	0 ±00	0 ±00	1 ±00	0.66 ±0.33	0.66 ±0.33	1 ±00	0.66 ±0.33	1 ±00	Gen=19±0.3
Salmonella typhi	3.66 ±0.3	3 ±1	3.33 ±0.88	3.66 ±2.33	7.6 ±0.88	00 ±0	0 ±00	0 ±00	0 ±00	1.5 ±0.16	Van= 6±0.6
Salmonella typhipara A	1.33 ±0.1	0.66 ±0.33	0.66 ±0.33	0.33 ±0.33	1.33 ±0.1	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Strep=13±0.7
Salmonella typhipara B	1.33 ±0.3	1.5 ±0.2	1.33 ±0.3	1 ±00	1.16 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Strep=13±0.9
Staphylococcus aureus	0.83 ±0.44	0.83 ±0.44	1 ±0.5	1 ±0.57	1.16 ±0.16	1 ±00	0.33 ±0.2	0.33 ±0.2	0.33 ±0.2	1 ±00	Pen = 4±0.4
MRSA	0.33 ±0.1	0.33 ±0.1	0 ±0	0± 00	0.83 ±0.3	1 ±00	1 ±00	1 ±00	1 ±00	1.33 ±0.33	Met = 6±0.32
Streptococcus fecalis	6.6 ±1.2	7.3 ±2.18	6.6 ±1.85	8 ±1	10.3 ±0.88	0 ±00	0 ±00	0 ±00	0 ±00	1 ±0.33	Nov = 17±00
Pseudomonas aeruginosa	2.5 ±0.76	2.1 ±0.16	1.6 ±0.33	1.6 ±0.33	1 ±0.66	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Van = 5±0.1
Escherichia coli	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	1.33 ±0.33	Van = 8±0.12
Klebsiella pneumonia	5± 0.5	2.6 ±0.3	2.6 ±0.3	4 ±0.57	4.6 ±0.66	0.5 ±0.2	0.33 ±0.2	0.33 ±0.2	0.33± 0.2	0.33 ±0.2	Amp= 12±0.5
Corynebacterium xerosis	1.3 ±0.16	1.5 ±0.28	2.3 ±0.72	1.6 ±0.33	2.3 ±0.33	1.5 ±0.2	1.33 ±0.3	2 ±0.2	1.5 ±0.2	3.33 ±0.16	Poly= 16±0.3
Staphylococcus epidermidis	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	1 ±00	1 ±00	1.16 ±0.16	1.16 ±0.16	1.33 ±0.33	Rif = 10±0.3
Staphylococcus saprophyticus	1 ±00	1 ±00	1 ±00	1 ±0.16	1 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Eryth=14±0.3
Bacillus subtilis	0.5 ±0.5	0.3 ±0.3	0.3 ±0.3	0.3 ±0.3	1.3 ±0.3	1 ±00	1 ±00	1 ±00	1.16 ±0.16	1.83 ±0.16	Nov = 16±00

Van=Vancomycin, Amp=Ampicillin, Gen=Gentamycin, Strep=Streptomycin, Pen=Penicillin, Met=Methicillin, Nov=Novobiocin, Poly=Polymixin, Rif=Rifampin, Eryth=Erythromycin

Methanol extract

No zone of inhibition was observed against *A.flavus, A.niger, Penicillium* species and *M.gypseum.* In case of *Candida* species, small zones were observed at all the concentrations except 5000ug, on which large zone was produced. The zones of inhibition against *Mucor* species and *S.cerevisae*, were small at all the concentrations. The zone of inhibition against *T.mentagrophytes* was larger at all the concentrations of extract as compared to the zone of nystatin. While at 4000ug, small zone of inhibition was observed (Table 2d).

Effect of stem and leaves extracts on bacterial isolates

K.pneumoniae, P.marbilis, S.dysenteriae, S.aureus, S.typhi, S.typhi para A, S.typhi para B, P.aeruginosa, E.coli, E.faecalis, S.saprophyticus, S.epidermidis, MRSA, B.subtilis, M.luteus, and *C.xerosis* were treated with methanol,

chloroform, ethyl acetate and hexane extracts of stem and leaves (Table 3a-d). It was observed that at all the concentrations of extracts used, the zones of inhibition were statistically significant (p <0.001***) as compared to the zone of inhibition of positive control antibiotics. Different antibiotics including streptomycin, gentamycin, vancomycin, ampicillin, polymixin, methicillin, erythromycin, rifampin, penicillin and novobiocin were used as positive control and DMSO as a negative control. In case of DMSO, no zone of inhibition was observed while in case of standard antibiotics, large zone of inhibition were observed and used to compare with the test extracts.

Table 3c.Antibacterial activity of Methanol extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	**Zone of inhibition at different concentrations (mm) STEM**					**Zone of inhibition at different concentrations (mm) LEAVES**					**Zone of inhibition (mm**
	5%	**10%**	**15%**	**20%**	**100%**	**5%**	**10%**	**15%**	**20%**	**100%**	
Proteus mirabilis	1.16 ±0.7	1 ±0.5	1.16 ±0.7	1 ±0.5	1.16 ±0.7	1.16 ±0.6	2 ±0.5	1.5 ±0.7	1.3 ±0.6	1.16 ±0.16	Van=8±0.3
Micrococcus luteus	1 ±00	1 ±00	1 ±00	1 ±00	2 ±0.57	1 ±0.5	1 ±0.5	1.5 ±0.2	2 ±0.2	2.3 ±0.3	Amp=10±0.4
Shigelladysentriae	0.66 ±0.33	1 ±00	1 ±00	0.66 ±0.33	1 ±00	00 ±0	0 ±00	0 ±00	0 ±00	0 ±00	Gen= 19±0.3
Salmonella typhi	0.6 ±0.33	0.6 ±0.33	0.6 ±0.33	0.3 ±0.2	1 ±00	1.13 ±0.33	1.16 ±0.16	1.16 ±0.16	1.16 ±0.16	1 ±00	Van = 6±0.6
Salmonella typhipara A	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	1.16 ±0.16	1.16 ±0.16	1.6 ±0.44	1.5 ±0.28	2 ±0.28	Strep=13±0.7
Salmonella typhipara B	0.3 ±0.33	0.3 ±0.33	0.3 ±0.33	0.3 ±0.33	1 ±0	1.3 ±0.16	1.16 ±0.16	1.6 ±0.44	1.5 ±0.28	2 ±0.28	Strep=13±0.9
Staphylococcus aureus	0 ±00	0 ±00	0 ±00	0 ±00	1.16 ±0.28	1.5 ±0.28	1.16 ±0.16	1 ±00	1.16 ±0.16	1.3 ±0.16	Pen = 4±0.4
MRSA	0.6 ±0.33	0.3 ±0.2	0.3 ±0.2	0 ±00	1 ±00	1.16 ±0.16	1.16 ±0.16	1 ±00	1 ±00	1.3 ±0.33	Met= 6±0.32
Streptococcus fecalis	1.33 ±0.66	1.33 ±0.66	1.5 ±0.76	2 ±00	2.1 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Nov = 17±00
Pseudomonas aeruginosa	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	1 ±00	0.6 ±0.3	0 ±00	0 ±00	1.5 ±0.28	Van = 5±0.1
Escherichia coli	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	1 ±00	0.6 ±0.33	0 ±00	0 ±00	1 ±00	Van= 8±0.12
Klebsiella pneumonia	0 ±00	0.6 ±0.3	0.6 ±0.3	1.1 ±0.16	1 ±00	0 ±00	0 ±00	0.66 ±0.33	1 ±00	1.16 ±0.16	Amp=12±0.5
Corynebacterium xerosis	0 ±00	0.6 ±0.33	0.6 ±0.33	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	1.16 ±0.16	Poly=16±0.3
Staphylococcus epidermidis	1.33 ±0.16	0.83 ±0.44	0.83 ±0.44	1.5 ±0.8	2.3 ±0.3	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Rif = 10±0.3
Staphylococcus saprophyticus	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Eryth=14±0.3
Bacillus subtilis	0 ±00	0 ±00	0 ±00	0 ±00	1.3 ±0.33	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Nov = 16±00

Van=Vancomycin, Amp=Ampicillin, Gen=Gentamycin, Strep=Streptomycin, Pen=Penicillin, Met=Methicillin, Nov=Novobiocin, Poly=Polymixin, Rif=Rifampin, Eryth=Erythromycin

DISCUSSION

In this study, the anti-inflammatory, antifungal and antibacterial effect of *Physorrhynchus brahuicus* was investigated for the first time. Various fungal and bacterial isolates were treated with four types of solvents. Hexane, chloroform, ethyl acetate and methanol were used by keeping the point in mind that different solvents have different solubility for polar and non polar compounds so that we could analyze the antimicrobial effect of various compounds. The anti-inflammatory activity of various extracts was analyzed and compared to the standard anti-inflammatory drug aspirin (Govindappa *et al.*, 2011). Methanol extract of leaves and stem showed maximum inhibition of albumin denaturation while chloroform extract of stem also showed inhibition but not considerable.

The chloroform extract of *Physorrhynchus brahuicus* leaves showed significant activity against *Penicillium* species and *S.cerevisae* while more inhibition was observed against *T.mentagrophytes* at the concentrations of 250ug, 5000ug and 3000ug as compared to the positive control antibiotic nystatin. The inhibition was also observed against *A.flavus*, *A.niger,Candida* species, *Mucor* species but not significant. The ethyl acetate extract showed larger and similar zones

as nystatin produced against *A.flavus* and *Mucor* species. The zone of inhibition was also considerable against *A.niger*. In case of *Candida* species and *S.cerevisae*, significant inhibition was not observed and against *Penicillium* species and *T.mentagrophytes* no inhibition was observed. The hexane extract of leaves showed larger inhibition as compared to the antibiotic nystatin against *S.cerevisae* and *T.mentagrophytes* at 5000ug and 250ug, 750ug, 1500ug & 5000ug concentrations. 4000ug and 5000ug concentrations showed similar inhibition as nystatin against *Candida* species and *A.flavus.* Non-significant inhibitory zone was observed in case of *A.niger* and *Mucor species* and no zone against *Penicillium species.* In case of *T.mentagrophytes*, the methanol extract showed large zone of inhibition at concentrations of 750ug, 3000ug & 1500 and larger inhibition at 250 & 5000 concentrations as compared to the inhibition by nystatin. Inhibition was also observed against *Candida species, Mucor species* and *S.cerevisae* but not significant and, no inhibition of *A.flavus, A.niger* and *Penicillium species. M.gypseum* did not showed sensitivity to any of the leaves extract used.

Table 3d. Antibacterial activity of Ethyl acetate extract of stem and leaves (Means of triplicate zones of inhibition in mm ± SEM).

Name of organisms	**Zone of inhibition at different concentrations (mm) STEM**					**Zone of inhibition at different concentrations (mm) LEAVES**					**Zone of inhibition (mm**
	5%	**10%**	**15%**	**20%**	**100%**	**5%**	**10%**	**15%**	**20%**	**100%**	
Proteus mirabilis	1 ±0	1.16± 0.16	0.66 ±0.33	0.66 ±0.33	0.66 ±0.33	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Van=8±0.3
Micrococcus luteus	1.16 ±0.16	1.5 ±0.5	1.33 ±0.33	1 ±00	1 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Amp= 10±0.4
Shigelladysentriae	1.16 ±0.28	1.5 ±0.28	1.66 ±0.16	1.5 ±0.28	1.8 ±0.33	0 ±00	0 ±00	0 ±00	0 ±00	0.66 ±0.33	Gen= 19±0.3
Salmonella typhi	1 ±0.57	0.83 ±0.6	0.83 ±0.6	1.33 ±0.88	1.33 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Van = 6±0.6
Salmonella typhipara A	0 ±00	0 ±00	0 ±00	0.66 ±0.33	1.33 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	1 ±0.33	Strep=13±0.7
Salmonella typhipara B	1.66 ±0.44	2 ±0.57	2 ±0.5	2 ±0.6	2 ±0.5	0.33 ±0.2	2 ±0.5	0.33 ±0.2	0 ±00	0.66 ±0.33	Strep=13±0.9
Staphylococcus aureus	1.16 ±0.16	1.5 ±0.16	1.33 ±0.33	1 ±0.44	1 ±0.28	0 ±00	0 ±00	0 ±00	0 ±00	0.66 ±0.33	Pen = 4±0.4
MRSA	1.16 ±0.16	1.16 ±0.16	1.3 ±0.16	1.33 ±0.16	1.16 ±0.16	0.33 ±0.2	0.33 ±0.2	0.33 ±0.2	0.33 ±0.2	1.16 ±0.16	Met = 6±0.32
Streptococcus fecalis	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 0±0	0 ±00	0 ±00	1 ±00	Nov = 17±00
Pseudomonas aeruginosa	0.66 ±0.33	0.66 ±0.33	0.83 ±0.44	1 ±00	1.16 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	0.66 ±0.33	Van = 5±0.1
Escherichia coli	1.66 ±0.3	1.8 ±0.44	1.33 ±0.16	1.33 ±0.16	1.5 ±0.28	0 ±00	0 ±00	0 ±00	0 ±00	0.83 ±0.4	Van = 8±0.12
Klebsiella pneumonia	1.33 ±0.33	1.16 ±0.16	1 ±00	1.5 ±0.5	1 ±0.4	1 ±0.5	1 ±0.5	1 ±0.5	1.16 ±0.6	1.83 ±0.44	Amp= 12±0.5
Corynebacterium xerosis	1 ±00	1 ±00	1.16 ±0.16	1 ±00	1.33 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	0.83 ±0.44	Poly= 16±0.3
Staphylococcus epidermidis	1.16 ±0.16	1.16 ±0.16	1.16 ±0.5	1.5 ±0.5	1 ±00	0 ±00	0 ±00	0 ±00	0 ±00	0 ±00	Rif = 10±0.3
Staphylococcus saprophyticus	2 ±00	2.5 ±0.5	2.5 ±0.5	2.33 ±0.3	2 ±0.2	1 ±00	0 ±00	0 ±00	0 ±00	1.16 ±0.16	Eryth=14±0.3
Bacillus subtilis	1.16 ±0.16	1.33 ±0.16	1 ±00	1 ±00	2.33 ±0.16	0 ±00	0 ±00	0 ±00	0 ±00	0.66 ±0.3	Nov = 16±00

Van=Vancomycin, Amp=Ampicillin, Gen=Gentamycin, Strep=Streptomycin, Pen=Penicillin, Met=Methicillin, Nov=Novobiocin, Poly=Polymixin, Rif=Rifampin, Eryth=Erythromycin

The chloroform extract of stem of *Physorrhynchus brahuicus* showed similar inhibition as nystatin against *Mucor species, Candida species* and *A.flavus* at 5000ug concentration. Less inhibition was observed in case of *A.niger, Penicillium species* and *S.cerevisae* and no zone against *T.mentagrophytes* and *M.gypseum.* The ethyl acetate extract of stem showed large zones of inhibition nearer to the zones of nystatin against *A.niger, Penicillium species and Candida species.* In case of *A.flavus, Mucor species, S.cerevisae* and *T.mentagrophytes*, significant inhibition was not observed and against *M.gypseum* no inhibition was found. In case of *T.mentagrophytes*, *Mucor species*, *S.cerevisae*, *A.niger* and *Candida species*, the hexane extract of stem showed similar and large zones of inhibition at

concentrations of 4000ug and 5000ug. Larger inhibition was observed against *A.flavus* as compared to the inhibition by nystatin at 1500ug, 3000ug, 4000ug and 5000ug concentrations and no inhibition was found in case of *M.gypseum* and *Penicillium species.* The methanol extract of stem showed larger zones of inhibition against *Mucor species* as compared to nystatin at 250ug, 4000ug and 5000ug concentrations. The inhibition was also observed against *A.flavus, A.niger,Candida species, S.cerevisae* and *Penicillium species* but not significant. No zone of inhibition was found against *M.gypseum* and *T.mentagrophytes.* The significant zones of inhibition were observed at 5000ug concentration of all the (leaves and stem) extracts against different fungal isolates. Non-significant difference between the zones of inhibition of the test extracts and nystatin showed that the extract has the activity closer to the activity of antibiotic nystatin.

In case of bacterial isolates, very less zones of inhibition were observed when they were treated with all extracts. Among all the concentrations used, none of them was able to produce good inhibition and could be nearer or similar to the inhibition by positive control antibiotics. This is showing that all the extracts we used have very less antibacterial activity.

CONCLUSION

These results showed that the leaves and stem of *Physorrhynchus brahuicus* plant have anti-inflammatory as well as potential antifungal activity. However, having very limited antibacterial activity. This study may support the ethnomedicinal use of the studied plant extracts. Further research is needed to isolate their constituents and to explore their individual mechanism of action so that these extracts can be used reliably.

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