International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 4 | OCT-DEC| 2017 | 133-138
Research Article | Biological Sciences | Open Access | UGC Approved | MCI Approved Journal

EVALUATION OF ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF SELECTED FUNGAL EXTRACTS

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ABSTRACT

The aim of the present study is to examine the antibacterial and anti-inflammatory activities of the fungal extract of 5 different strains (Cladosporium sp., Trichoderma sp., Fusarium sp., Helminthosporium sp., were used. It was tested in vitro for its antibacterial and anti-inflammatory activities against different pathogenic bacteria's (Staphylococcus sp., Micrococcus sp., Escherichia coli, Bacillus subtilis sp., and Pseudomonas sp). Significant results were showed in Trichoderma sp., exhibits the good inhibitory activity against all the five such as Staphylococcus sp., Micrococcus sp., Escherichia coli, Bacillus subtilis sp., and Pseudomonas sp. Our investigation pointed that fungal extracts could be considered as a good medicinal agent. In conclusion some secondary metabolites of fungi have a promising potential as antibacterial and anti-inflammatory compounds. The present investigation was an attempt to search for antibacterial and anti-inflammatory pattern from an alternate source from fungus.

KEY WORDS

Fungi; Antimicrobial; Anti-inflammatory; Drug discovery.

INTRODUCTION

Nature is an attractive source of new therapeutic candidate compounds since a tremendous chemical diversity is found in millions of species of plants, animals, marine organisms and microorganisms. Microorganisms such as bacteria and fungi have been invaluable to discover drugs and lead compounds. These microorganisms produce a large variety of antimicrobial agents which have evolved to give their hosts an advantage over their competitors in the microbiological world. For example: the fungal metabolite lovastatin, which was the lead compound for a series of drugs that lower cholesterol levels, the cyclosporin (fungal metabolite) currently used to suppress the immune response after transplantation operations and sirolimus- a bacterium-derived macrolide- used in the treatment of some cancers. [1]

Since humans (animals) and fungi share common microbial antagonists such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, humans can benefit from the natural defensive strategies of fungi that produce antibiotics to fight infection from microorganisms. The fact that mushrooms can have both anti-viral and anti-bacterial properties, with low cytotoxicity to animalian hosts, underscores their usefulness as natural sources of medicine. [2]

Fungal endophytes produce various kinds of secondary metabolites, which have bioactive potential such as antioxidant, anti-inflammatory, anti-diabetic, anti-tumor, antimicrobial properties. [3] A large number of fungal bioactive compounds, both cellular components and secondary metabolites, have been shown to affect the human immune system and could be used to treat a variety of diseases. [4] A large number of fungal bioactive compounds, both cellular components and secondary metabolites, have been shown to affect the human immune system and could be used to treat a variety of diseases [5]. Recent intensification in the application of bioactive compounds produced by white

rot fungi in the food processing or pharmaceutical industry is stimulating a worldwide search for new natural bioactive compounds of fungal origin. [6] A number of these substances including intra- and extracellular low molecular weight compounds, proteins, polysaccharides, or polysaccharide-protein complexes have been isolated from wood-degrading fungal strains. [7]

Inflammation is a complex biological response of vascular tissues to harmful stimuli. It is also a protective attempt by the organism to remove the injurious stimuli and initiate the healing process. [8] At the onset of an inflammation, the cells undergo activation and release inflammatory mediators. These mediators include histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), prostaglandins and some plasma enzyme systems such as the complement system, the clotting system, the fibrinolytic system and the kinin system. [9] These mediator molecules work collectively to cause increased vasodilatation and permeability of blood vessels. Thus, leading to increased blood flow, exudation of plasma proteins and fluids, and migration of leukocytes, mainly neutrophils, outside the blood vessels into the injured tissues. [10]

By taking the all above considerations, the present investigation was an attempt to search for antibacterial and anti-inflammatory pattern from an alternate source from fungus. The study revealed the presence of good antibacterial and anti-inflammatory activities for the fungal extracts of *Trichoderma viride, Fusarium oxysporum, Cladosporium cladosporioides* and *Helminthosporium species.* So, the *Trichoderma* could be a good source for bioactive and as an antibacterial and anti-inflammatory agent.

2. MATERIALS AND METHODS

2.1 Isolation of fungal metabolites:

The fungal cultures of selected strains *Trichoderma viride, Fusarium oxysporum, Cladosporium cladosporioides, Helminthosporium* were subculture on 5 different types of broth media (PDB, NB, Sabouraud's, Emmon's, and Dextrose media) in the 250ml conical flask containing 100ml of the media's and placed on a thermostatic shaker with the rotary speed of 180 rpm at 28°C for 7 days for fermentation. The grown fungi was separated and Homogenized and filtered through Whatman filter paper no.1 and stored in large test tubes under 5°C. [11]

2.2 Antibacterial activity:

In vitro antibacterial screening of selected fungal was carried out against five pathogenic strains, *Escherichia coli*, Staphylococcus *aureus*, Pseudomonas *aeruginosa*, and *Bacillus subtilis* by disc diffusion method. [12]

20 ml of sterilized Nutrient Agar was poured into sterile petri plates, after solidification of the media, pour 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The disc was loaded with 30µl of the fungal extract and 10µl of ampicillin as control. Loaded discs were kept over the agar plates using sterile forceps. The plates were incubated for 24 hours at 37ºC. Plates were then examined for growth inhibition zones, and the diameters were measured, the experiments were carried out in triplicates. [13]

2.3 *In vitro* anti-inflammatory activity

2.3.1 HRBC membrane stabilization assay

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defence and usually activated in most disease condition. Blood was collected freshly and mixed with equal volume of Alsever solution. It was then centrifuged at 3000 rpm for 15 minutes. The packed cells were washed with isosaline and a 10% suspension was made with isosaline. To 0.5 ml of extract, 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml HRBC (Human red blood cell) suspension were added. This was incubated for 30 minutes at 37 ºC and then centrifuged at 3000 rpm for 20 minutes. Absorbance was measured at 560 nm. Control was taken without the extract. [14]

Statistical analysis

The results were expressed as mean ± SD of three independent experiments ($P<0.01$).

3. RESULTS AND DISCUSSION

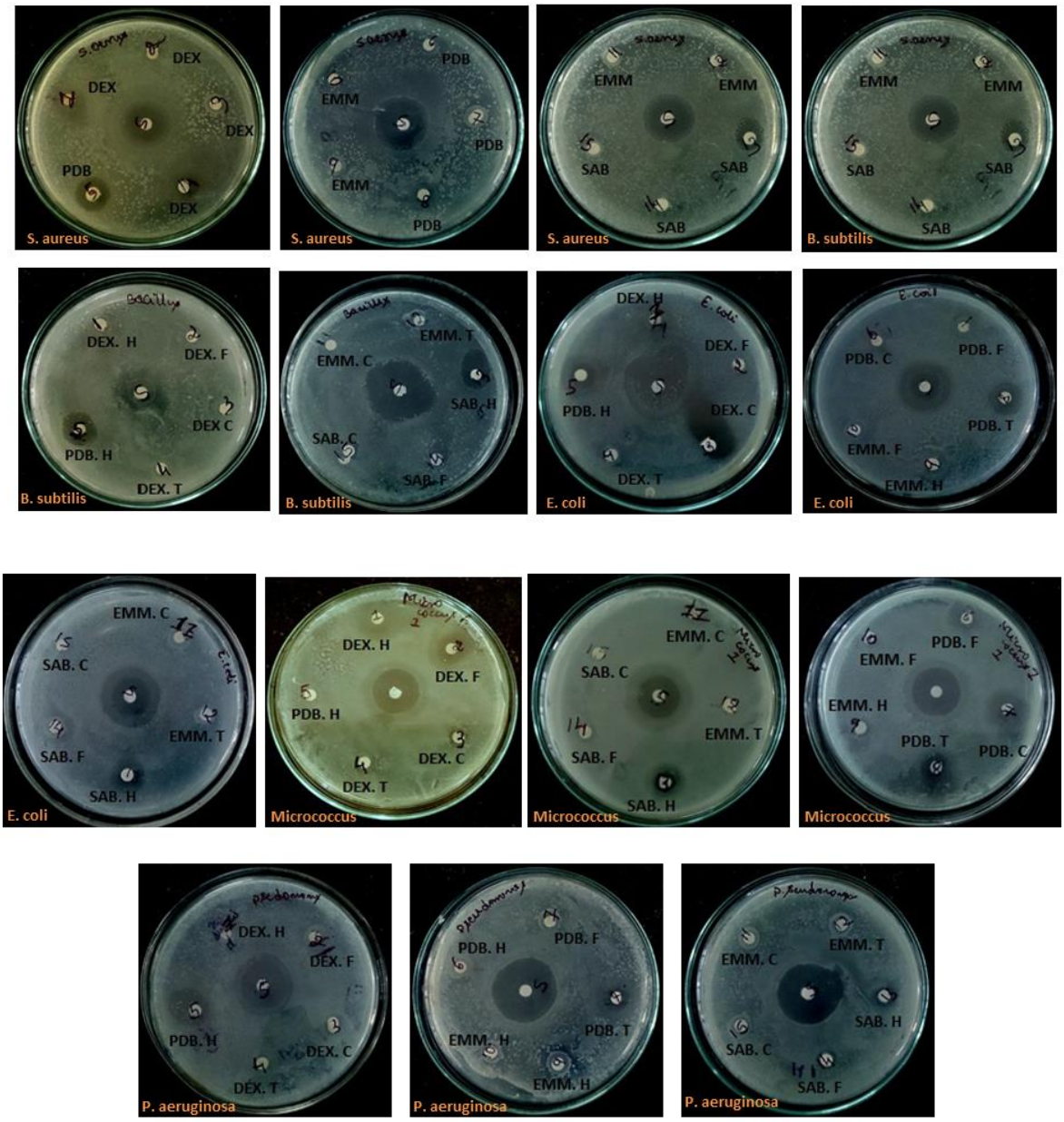
3.1 Antibacterial activity of Fungal extracts:

Four fungal extracts evaluated for antibacterial activity, 30µl of the fungal extract and 10µl of amphicillin used as control is showed in (Fig.1). Among the four fungal extracts evaluated for antibacterial activity, The *Trichoderma viride* extract showed maximum inhibitory activity against all five bacterial strains is showed in (Fig. 2 and Table. 1). The zone of inhibition is measured in mm. The plates were incubated at 37^0C for 24hr and the

resulting zone of inhibition was measured by comparing to the control and the reference antibiotic (amphicillin).

Four fungal extracts evaluated for antibacterial activity, 30µl of the fungal extract and 10µl of ampicillin used as control.

Fig 1: Anti-bacterial activity of Fungal extracts



DEX: Dextrose broth
PDB: Potato dextrose Broth
EMM: Emmons broth
SAB: Sabourauds Broth

H: *Helminthosporium*
F: *Fusarium*
C: *Cladosporium*
T: *Trichoderma*

Table 1: Antibacterial activity of selected fungal extracts:

Microorganisms	Zone of inhibition in mm				
	Antibiotic 10µg	*Helminthosporium* 20µg	*Fusarium* 20µg	*Cladosporium* 20µg	*Trichoderma* 20µg
Micrococcus	22 ± 1.0	19 ± 1.0	12.6 ± 0.2	6.4 ± 0.4	10.83 ± 0.5
E.coli	21 ± 0.8	0	0	15.6 ± 0.5	19.5 ± 0.7
S.aureus	21 ± 1.0	0	0	0	14.4 ± 0.4
B.subtilis	23 ± 0.4	17.66 ± 0.5	0	0	9.1 ± 1.0
P. aeruginosa	22 ± 0.7	0	0	8.3 ± 0.5	10.4 ± 0.4

The results are expressed as mean ± SD (n=3)

Fig 2: Antibacterial activity of selected fungal extracts:

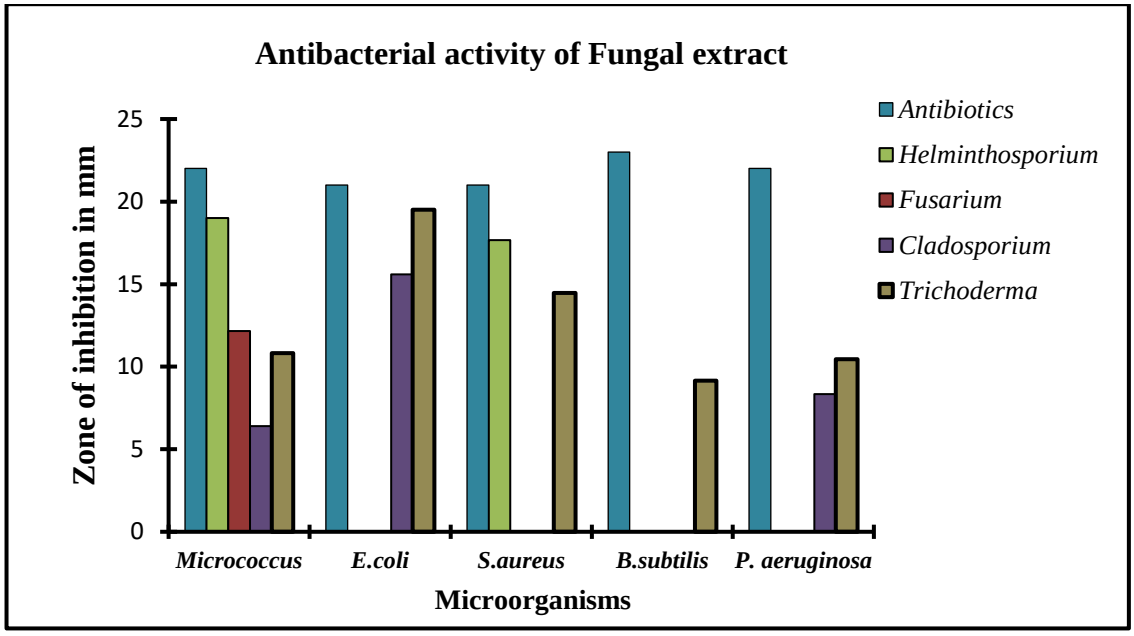
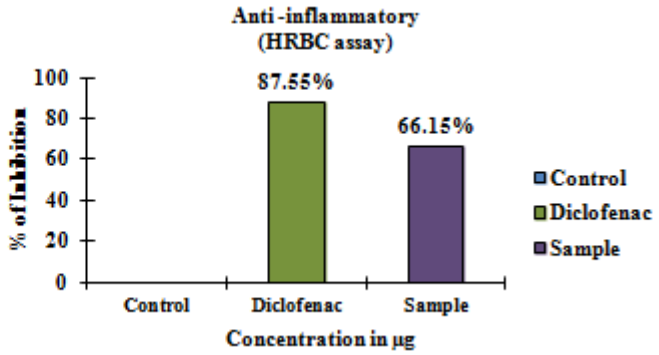


Fig 3: Anti-inflammatory activity selected fungal extracts:

Name	Concentration in µg	% of Inhibition
Control	---	0 ± 0.0
Diclofenac	100	87.55 ± 0.41
Sample	100	66.15 ± 0.30



3.2 Anti-inflammatory activity

3.2.1 Human Red Blood Cell membrane stabilization (HRBC) Assay:

Diclofenac was used as reference standard and 2ml of distilled water was used in the control. The Column purified *T. viride* sample exhibit considerable anti-inflammatory activity. The sample 200µg/ml showed 66.15% of inhibition, while Standard Diclofenac 100µg/ml showed 86.15% of inhibition is showed in (Fig. 3).

In recent years, the search for possessing antibacterial and anti-inflammatory properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Due to risk of adverse effects encountered with the use of synthetic antibiotics, in addition, a number of antibiotics have lost

their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes [15]. A large number of fungal bioactive compounds; both cellular components and secondary metabolites have been shown to affect the human immune system and could be used to treat a variety of diseases. [16] In our research the fungal extracts of four different strains were sub cultured on 5 different types of broth media (PDB, NB, Sabouraud's, Emmon's, and Dextrose media) and performed the antibacterial assay against the five pathogenic strains. Significant results were showed in *T. viride*; exhibits the good inhibitory activity against all the five selected pathogenic bacteria. [13]

Stabilization of the RBCs membrane was studied to further establish the mechanism of anti-inflammatory of action of *T. viride* purified fungal Extract. The extracts exhibited membrane stabilization effects by inhibiting hypo tonicity induced lyses of erythrocyte membrane. [17. Stabilization of lysosomal membrane is important in limiting the inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases. [18] *T. viride* extract may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation, which may be due to presence of chemical profile such as flavones, terpenoids, tannins and phenols.

4. CONCLUSION

The present investigation was an attempt to search for antibacterial and anti-inflammatory pattern from an alternate source from fungus. The study revealed the presence of good antibacterial and anti-inflammatory activities for the crude and column purified extracts of *Trichoderma species.* So the *T. viride* could be a good source for bioactive and as an antibacterial agent. Further the mechanism of action of this purified extract has to be thoroughly scrutinized for its uses as therapeutic drug.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGMENT

The author sincerely thanks to the authorities of JSS Mahavidyapeetha, Mysuru and Principal of JSS College of Arts, Commerce and Science (Autonomous), B N. Road Mysuru-25, Karnataka and India for providing laboratory facilities and constant support.

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