Phytochemical Screening, Antimicrobial and in vitro Anti-inflammatory Activity of Endophytic Extracts from *Loranthus* sp.

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ABSTRACT

Four different endophytes were isolated from different parts of *Loranthus* sp. Methanol and water extracts of all the endophytes was assessed for its antimicrobial and anti-inflammatory activity and phytochemical screening. Phytochemical analysis revealed the presence of tannins, flavonoids, terpenoids, steroids, alkaloids, phenols and saponins. The antimicrobial efficacy was determined using paper disc diffusion method against different fungi and bacteria. Sensitivity in terms of zones of inhibition and phytochemical composition of the all endophytic extracts were also determined. The results show that, *A. niger*, *Penicillium* sp. and *Alternaria alternata* extracts effective against all the bacteria and fungi tested, whereas *A. flavus* extract was failure in inhibiting the growth of all bacteria and bacteria. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation, membrane stabilization assay and proteinase inhibitory assay. Aspirin was used as a standard drug for the study of anti-inflammatory activity. *A. niger*, *Penicillium* sp. and *Alternaria alternata* methanol fractions showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation (87.88, 86.89, 87.03 g/ml) and red blood cells membrane stabilization with 78.42, 77.61, 77.98 g/ml respectively. Proteinase activity was also significantly inhibited by the *A. niger* (85.21 g/ml), *Alternaria alternata* (84.09 g/ml) and *Penicillium* sp. (79.17 g/ml). BSA anti-denaturation and HRBC membrane stabilization assay indicated that the methanol extracts of *A. niger*, *Penicillium* sp. and *Alternaria alternata* possess constituents with anti-inflammatory properties. From the result, it is concluded that phytochemicals (tannins, flavonoids, terpenoids, phenols, steroids, alkaloids and saponins) present in the *A. niger*, *Penicillium* sp. and *Alternaria alternata* extract may be responsible for the antimicrobial and anti-inflammatory activity.

Key words: endophytes, antimicrobial, anti-inflammatory, phytochemicals, *Loranthus* sp.

INTRODUCTION

The increase in prevalence of multiple drug resistance has showed down the development of new synthetic antimicrobial, anti-inflammatory drugs and the new drug is necessary to search for new antimicrobial, antioxidant and anti-inflammatory from alternative sources. Phytochemicals from medicinal plants showing antimicrobial, antioxidant and anti-inflammatory activities have the potential of filling this need because of structures are different from those of the more studied and their of the more action may too very likely differ.[1] In this growing interest, many of the Phytochemical bioactive compounds from a medicinal plants have shown many pharmacological activities.[2,3] Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles in against various diseases.[4] The rapid emergence of multiple drug resistance strains of pathogens to current antimicrobial agents has generated an urgent intensive for new antibiotics from medicinal plants. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide.[5,6] Endophytic fungi are relatively unexplored producers of metabolites useful to pharmaceutical and agricultural industries.[7] Endophytes are the microorganisms that grow inside the plants; both
(plant and endophytes) will be beneficial. Fungal endophytes residing within these plants could also produce metabolites similar to or with more activity than that of their respective hosts.\(^8\) Microorganisms are a rich source of biologically active metabolites that find wide-ranging exploitation in medicine, agriculture and industry.\(^9\) Many of the anticancer agents are explored from endophytes rather than host (taxol from \textit{Pestalotiopsis microspora}).\(^10\) Various research groups have identified more than hundreds of endophytic isolates from South Indian medicinal plants that showed promising activity against antitumour and antimicrobial agents.\(^11,12\)

The development of drug resistance in human and pathogenic bacteria and fungi has prompted a search for more and better antibiotics, especially as disease caused by pathogenic microorganisms, now represents a clear and growing threat to world health.\(^13,14\) Many of the endophytic fungal strains have attracted special attention because they have the capability of producing different colored pigments with high chemical stability. Globally, there are at least one million species of endophytic fungi in all plants,\(^15\) which can potentially provide a wide variety of structurally unique, bioactive, natural products.\(^16,17\)

Increasing evidence indicate that Reactive Oxygen Species (ROS), (example, \(O_2^-\) and \(OH^-\)) and free radical mediated reactions can cause oxidative damage to biomolecules (for example, lipids, proteins and DNA), eventually contributing to; aging, cancer, atherosclerosis, coronary heart ailment, diabetes, Alzheimer’s disease and other neurodegenerative disorders.\(^18,19\)

\textit{Loranthus} is a genus of parasitic plants that grow on the branches of woody trees. It belongs to the family Loranthaceae (the showy mistletoe family). \textit{Loranthus micranthus} exhibited various degree of antimicrobial\(^20\) and antidiabetic activity.\(^21\) \textit{L. europaeus} have showed hematopoietic activity.\(^22\)

The literature survey indicates that no reports are available from India regarding antimicrobial and anti-inflammatory activity of \textit{Loranthus} sp. endophytic extract. The present study was aimed to examine the total phenolic content and phytochemical analysis of water and methanol extract endophytes of \textit{Loranthus} sp. were screened for antimicrobial and anti-inflammatory properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the plant.

**MATERIALS AND METHODS**

The plant was collected in November 2010 from our college campus (Shridevi Institute of Engineering & Technology, Sira Road, Tumkur, Karnataka, India). The plant was identified by their vernacular names and later it was compared with the herbarium of Department of Studies in Botany, Manasa Gangothri, University of Mysore, Mysore and Government Ayurvedic College, Mysore, India.

**Isolation and identification of endophytic fungi**

The protocol for isolation follow methods used in other endophyte study\(^23\) but adjusted for the specific plant tissues used here following pilot experiments. The plant tissues were washed in running tap water for one hour. Fifty segments of leaves from each plant were cut into 5 mm 2 pieces, including a vein (25 samples) and intervein (25 samples). 25 segments of branches were then cut randomly to a length of 5 mm. Endophytic fungi were isolated from the bark of the plant (25 segments). Twenty five segments (5 mm long) were cut from the stems and the roots. The total 150 segments of plant material were treated by triple surface sterilization technique.\(^24\) Each piece was then placed on malt extract agar (malt extract (20 g/l), rose Bengal (0.033 g/l), chloromphenicol (50 mg/l, agar (15 g/l). All plates were incubated at 26 ± 2 °C until mycelium grew out hyphal tips were cut and transferred to Potato Dextrose Agar (PDA). Half strength PDA was used for subculture and stock culture. Identification was based on colony and hyphal morphology of the fungal cultures, characteristics of the spores,\(^25,26\)

**Fungal cultivation and extraction of metabolites**

The fungal endophytes were cultivated on Potato Dextrose Broth (Himedia, Germany) by placing agar blocks of actively growing pure culture (3 mm diameter) in 250 ml Erlenmeyer flasks containing 100 ml of the medium. The flasks were incubated at 26 ± 2 °C for 1 week with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out and filtered through sterile cheesecloth to remove the mycelia mats.

**Solvents**

Identification of the phytochemical active substances carried out using methanol solvent at 5 g/15 ml (W/V).

**Phytochemical analysis**

Chemical analysis was carried out in the methanol and water extracts of the all endophytes of \textit{Loranthus} sp. using standard procedures to identify constituents, as described by Harborne (1984), Trease and Evans (1979) and Sofowara (1993).\(^27,28,29\)

**Determination of antimicrobial activity**

**Antimicrobial assay**

\textit{Bacillus subtilis}, \textit{Pseudomonas fluorescens}, \textit{Clavibacter michiganensis} sub sp. \textit{michiganensis}, \textit{Xanthomonas oryzae} pv. \textit{oryzae}, \textit{Xanthomonas oryzae} pv. \textit{oryzae} strain of \textit{Staphylococcus aureus}, \textit{E. coli}, \textit{Pseudomonas aeruginosa} and \textit{Klebsiella pneumonia} bacteria were obtained from stock cultures presented at –80 °C at
Department of Studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of Studies in Biotechnology and Microbiology, Bangalore University, Ganna Bharathi, Bangalore, India respectively. Three Gram positive bacteria tested were Bacillus subtilis, Clavibacter michiganensis sub sp. michiganensis, Staphylococcus aureus and six Gram negative bacteria tested were Pseudomonas fluorescens, Xanthomonas oryzae pv. oryzae, Xanthomonas campestris pv. malvaearum, E. coli, Pseudomonas aeruginosa and Klebsiella pneumonia. All bacteria were grown on nutrient agar media.

Fungi (Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, Aspergillus flavipes, Alternaria carthami, Alternaria helianthi, Ceratocystis carthami, Fusarium solani, Fusarium oxysporum, Fusarium verticillioides and Nigrospora oryzae were obtained from Department of studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of studies in Microbiology, Bangalore University, Ganna Bharathi, Bangalore, India respectively. All fungi were grown on potato dextrose agar medium.

**Paper disc method**

Diameter of zone of inhibition was determined using the paper disc diffusion method as described by Lai et al. (2009) and Adedapo et al. (2008). A swab of the bacteria suspension containing 1 x 10^8 cfu/ml was spread on to the plates containing nutrient agar media. Each extracts were dissolved in ethanol to final concentration of 10 mg/ml. Sterilized filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37 °C for 24 h. The methanol served as negative control while the standard streptomycin (10 μg) discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

**In vitro anti-inflammatory activity**

**Inhibition of albumin denaturation**

Methods of Mizushima and Kobayashi (1968) and Sakat et al. (2010) followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37 °C HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min. after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows,

\[
\% \text{ inhibition} = \left[ \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}} {\text{Abs}_{\text{control}}} \right] \times 100,
\]

Where \(\text{Abs}_{\text{control}}\) is the absorbance of the DPPH radical+ solvent, \(\text{Abs}_{\text{sample}}\) is the absorbance of DPPH radical+ sample extract/standard.

**Membrane stabilization test**

**Preparation of red blood cells (RBCs) suspension**

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.[33]

**Heat induced hemolytic**

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicate for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned above.[33]

**Protein inhibitory action**

The test was performed according to the modified method of Oyedepo and Femurewas (1995) and Sakat et al. (2010). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

**BSA anti-denaturation assay**

Five ml of each extract was dried in vacuum oven and redissolved in 5 ml of isosalone. Then, 1 mg/ml of all extracts were made from the abovementioned stock solution. To 1.8 ml of 1% of BSA solution, 0.2 ml of extract solution in isosalone was added. The pH was adjusted to 6.5 using 1N HCl. This solution was incubated at 37 °C for 20 minutes and then heated to 57 °C for 10 to 15 minutes. After cooling, turbidity was measured at 660 nm. Control was taken without the extracts.[33]
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HRBC membrane stabilization assay
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 suspension were added. This was incubated for 30 minutes at 37 °C and then centrifuged at 3000 rpm for 20 minutes. Absorbance was measured at 560 nm. Control was taken without the extract.[16]

Statistical analysis
Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups \( (p < 0.05) \). Means between treatment groups were compared for significance using Duncan’s new Multiple Range post test.

RESULTS

Phytochemical analysis
Loranthus sp. was collected from neem plants (Figure 1). All the incubated parts exhibited the presence of four different endophytic fungal species viz., Aspergillus niger, Aspergillus flavus, Penicillium sp. and Alternaria alternata (Table 1). In the phytochemical screening of endophytes, Aspergillus flavus has showed only presence of carbohydrates and cardio glycosides in methanol extracts whereas no phytochemicals was observed in water extracts. Other three endophytic extracts yielded all the phytochemicals in both methanol and water extracts viz., carbohydrates, tannin, steroids, cardiac glycosides, flavonoids, terpenoids, alkaloids, phenol, saponins and anthraquinones (Table 2).

Antimicrobial assay
The antimicrobial activities of methanol and water extracts of endophytes of Loranthus sp. gave different zones of inhibition on the organisms tested (Table 3). The ethanol Aspergillus niger, penicillium sp. and Alternaria alternata extract inhibited the growth of all most all the bacteria and fungal species significantly. E. coli, Pseudomonas fluorescens, Xanthomonas oryzae pv. oryzae, A. helianthi and Cercospora carthami are inhibited by methanol extract of Aspergillus flavus minimally, in water extracts there is no activity against all the bacteria and fungi (Table 3).

Anti inflammatory properties
Inhibition of albumin denaturation
Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation (Table 4). Maximum inhibition 87.88% was observed from methanol A. niger extract.

Table 1: List of endophytes from different parts of Loranthus sp on PDA media

<table>
<thead>
<tr>
<th>Types of endophytes</th>
<th>Leaves</th>
<th>bark</th>
<th>stem</th>
<th>root</th>
<th>petiole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vein</td>
<td>inter-vein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Experiments were repeated for thrice for each sample, + = presence

Figure 1: A: Loranthus sp on Neem plant, B: Flowers of Loranthus sp. C: Different endophytes from different parts of Loranthus sp.
followed by Penicillium sp. (86.89%) and Alternaria alternata (87.03%). Aspirin, a standard anti-inflammation drug showed the maximum inhibition 76.69% at the concentration of 200 μg/ml. In water endophytic extract, maximum inhibition 77.33% was observed from A. niger followed by Penicillium sp. (76.54%) and Alternaria alternata (77.21%). (Table 4).

**Membrane stabilization test**

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different methanol and water extracts of different endophytes. All the extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly

<table>
<thead>
<tr>
<th>Tests</th>
<th>Methanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

Experiments were repeated for thrice for each sample, +ve: positive, –ve: negative, 1- Aspergillus flavus, 2- A. niger, 3- Penicillium sp., 4- Alternaria alternata

**Table 3: In vitro inhibition assay from methanol and water extracts of endophytes**

<table>
<thead>
<tr>
<th>Species</th>
<th>Methanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial pathogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Clavibacter michiganensis sub sp. michiganensis</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Xanthomonas oryzae pv. oryzae</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Xanthomonas axanopodis pv. malvacearum</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Fungal pathogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>A. niger</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>A. flaviceps</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Alternaria carthami</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>A. helianthi</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Cercospora carthami</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>F. verticillidoides</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Nigrospora oryzae</td>
<td>++</td>
<td>–</td>
</tr>
</tbody>
</table>

++ = average, + = minimum activity, – = No activity, 1- Aspergillus flavus, 2- A. niger, 3- Penicillium sp., 4- Alternaria alternata, Repeated the experiments three times for each replicates

**Table 4: Effect of methanol and water extracts of different endophytes on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition**

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Albumin denaturation</th>
<th>Membrane stabilization</th>
<th>Proteinase inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>87.88 ± 0.006a</td>
<td>78.42 ± 0.03a</td>
<td>85.21 ± 0.03a</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>44.76 ± 0.006a</td>
<td>54.29 ± 0.03a</td>
<td>53.34 ± 0.03a</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>86.89 ± 0.006c</td>
<td>77.61 ± 0.03a</td>
<td>79.17 ± 0.03a</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>87.03 ± 0.006a</td>
<td>78.41 ± 0.03a</td>
<td>85.21 ± 0.03a</td>
</tr>
<tr>
<td>Water extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>77.33 ± 0.006a</td>
<td>72.54 ± 0.03a</td>
<td>82.04 ± 0.03a</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>39.41 ± 0.006a</td>
<td>54.81 ± 0.03a</td>
<td>50.19 ± 0.03a</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>76.54 ± 0.006c</td>
<td>71.87 ± 0.03a</td>
<td>77.89 ± 0.03a</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>77.21 ± 0.006a</td>
<td>71.95 ± 0.03a</td>
<td>81.86 ± 0.03a</td>
</tr>
<tr>
<td>Aspirin (200μg/ml)</td>
<td>75.89 ± 0.006c</td>
<td>85.92 ± 0.03a</td>
<td>92.87 ± 0.05a</td>
</tr>
</tbody>
</table>

Repeated the experiments three times for each replicates, According to Duncan’s Multiple Range Test (DMRT), values followed by different subscripts are significantly different at \( P \leq 0.05 \), SE-standard error of the mean.

The inhibitory effect on protein (BSA) denaturation by the water and methanol extracts of endophytes is shown in Figure 2. All the extracts were tested at 200 μg/ml concentration. The A. niger, Penicillium sp. and Alternaria alternata water and methanol fractions showed good activity, whereas the A. flavus extract showed comparatively lower activity. At 200 μg/mL concentration, A. niger methanol extract showed 79% inhibition of denaturation followed by Alternaria alternata (78.6%) and Penicillium sp. (65.84%). The water endophytic extracts also showed significant inhibition of denaturation by A. niger (76%), Alternaria alternata (76.1%) followed by (75.3%) (Figure 3).

### Proteinase inhibitory activity

The different endophytic ethanol extract exhibited significant antiproteinase activity. The maximum inhibition was observed from methanol A. niger extract (85.21%), in decreasing order was Penicillium sp. (79.17%) and Alternaria alternata (84.09%). The standard aspirin (92.87%) drug showed the maximum proteinase inhibitory action. In water endophytic extract, maximum inhibition 82.04% was observed from A. niger followed by Penicillium sp. (77.89%) and Alternaria alternata (81.86%)(Table 4).
systemic lupus erythematosus. Thus, this assay was applied for the detecting compounds, which can stabilize the protein from denaturation process. Several nonsteroidal anti-inflammatory drugs such as Indomethacin, Ibuprofen, Diclofenac sodium, salicylic acid and flufenamic acid prevent denaturation of BSA at pathological pH (6.2-6.5).[38]

**HRBC membrane stabilization assay**

After the initial screening of endophytes, it was found that the methanol extract showed activity similar to Diclofenac, the standard anti-inflammatory drug used, for treating inflammation. Various endophytic methanol extracts in isosaline were tested and it was observed at 250 μg/ml both Diclofenac and the endophytic extracts showed similar effects (Table 5). The analogous activity makes the extract a potential candidate for further studies.

**DISCUSSION**

In recent years, the search for phytochemicals possessing antimicrobial and anti-inflammatory properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc.[39] Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes.[40]

Results of our findings confirmed the use of endophytes, *A. niger*, *Penicillium* sp. and *Alternaria alternata* as traditional medicine. We found strong antimicrobial and anti-inflammatory activities specifically in the methanol extract of *A. niger*, *Penicillium* sp. and *Alternaria alternata*. Plant phenolic compounds have been found to possess potent antimicrobial[41,42] and anti-inflammatory activity.[33,43]

The flavonoids from extracts have been found to possess antimicrobial and anti-inflammatory properties in various studies.[44,45] The presence of terpenoids have shown as antimicrobial[46] and anti-inflammatory properties.[47]

Strong presence of tannins in all extracts may explain its potent bioactivities are known to possess potent antimicrobial activities[41] and anti-inflammatory properties.[48] The Saponins have already shown as antimicrobial activity[49] and anti-inflammatory activity.[50]

**In vitro anti inflammatory properties**

Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation.[42] Similar results were observed from many reports from plant extract.[33] The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage.[51] The precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the endophytes, *A. niger*, *Penicillium* sp. and *Alternaria alternata* of *Loranthus* sp. produced this effect surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins.[52]

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors.[53] Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the antioxidant and anti-inflammatory activities of many plants. Hence, the presence of bioactive compounds in the methanol extracts of different endophytes, *A. niger*, *Penicillium* sp. and *Alternaria*
**alternata** of *W. trilobata* may contribute to its, antimicrobial and anti-inflammatory activity.

The present investigation has shown that the *A. niger*, *Penicillium* sp. and *Alternaria alternata* extracts have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The methanol extract fractions showed significantly antimicrobial activity against all Gram-positive and Gram-negative bacteria and different fungi tested. Strong anti-inflammatory properties were confirmed in the methanol endophytic extract fractions. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols and saponins. The anti-inflammatory activity was comparable with standard ascorbic acid, BHT and aspirin. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial and anti-inflammatory agent from *A. niger*, *Penicillium* sp. and *Alternaria alternata*. These endophytes by *in vitro* results appear as interesting and promising and may be effective as potential sources of novel antimicrobial and anti-inflammatory drugs.

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