Research Article

In vitro Antioxidant and Anti-Inflammatory Activity of *Dendrophthoe falcata* Endophytic Fungal Ribosome Inactivating Proteins

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ABSTRACT

Aim: The goal of the current investigation was to characterize endophytic fungal Ribosome Inactivating Protein (RIPs) and these RIPs evaluated to analyse potentiality of antioxidant and anti-inflammatory property. **Materials and Methods:** Total five different endophytic fungi were subjected to identification of molecular weight by SDS-PAGE, PAS staining for glycoprotein identification and characterization of RIP by hemaglutination assay. Those RIPs evaluated for antioxidant (DPPH and FRAP) and anti-inflammatory activity (albumin denaturation study. anti-proteinase and anti-lipoxygenase assay). **Results:** 5 distinct types of endophytic fungus were isolated from the stem and leaves of *Dendrophthoe falcata* growing on *Lagerstroemia speciosa*. Obtained five endophytic fungal protein extracts exhibited the presence of glycoproteins with molecular weight 68 kDa. All endophytic fungal proteins are confirmed as glycoproteins in PAS staining, showed positive test for hemagglutination assay. The CcRIP and DpRIP show positive test for the erythrocytes of A, B and AB and all endophytes crude RIP also expressed the hemagglutination activity and it was depending on different blood groups. The CcRIP expressed the highest antioxidant activity in DPPH method followed by DpRIP, DpsRIP, CsRIP and DpRIP. In the FRAP assay, CcRIP and DpRIP protein extract exhibited significant antioxidant property. The CcRIP shows significant anti-inflammatory activity in all the three different methods (albumin denaturation study, anti-proteinase and anti-lipoxygenase assay). **Conclusion:** The study demonstrates that endophytic fungal RIPs are the potential sources of novel antioxidant and anti-inflammatory products.

Keywords: *Dendrophthoe falcata,* Endophytic fungi, Ribosome inactivating protein, Antioxidant, Anti-inflammatory.

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INTRODUCTION

Endophytic fungi are well known for their therapeutically important and chemically diverse secondary metabolites including alkaloids, flavonoids and lectins.[1] They are a diverse group of eukaryotic microorganisms and are a subject of interest for their potential Biotechnological applications. Among the many bioactive compounds produced by fungi, glycoproteins-particularly those with cytotoxic, antioxidant and anti-inflammatory properties, have gained significant attention due to their therapeutic applications.[2] Glycoproteins are versatile class of proteins that can bind to carbohydrates in a reversible manner and noted for their role in agglutination.

They moderate many processes including cell signalling and differentiation.[3] Ribosome inactivating proteins are one such a class of proteins that can attach themselves to certain sugar compounds on the surfaces of cells and have been shown to exhibit potent anti-cancer activities.[4] They stop tumour development and metastasis by causing cancer cells to undergo autophagy, apoptosis and cell cycle arrest.[5]

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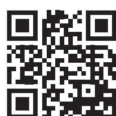

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Ribosome Inactivating Proteins (RIPs) are cytotoxic proteins, which resemble N-glycosidases, by eliminating one adenine from the rRNA's generally conserved sarcin-ricin loop, thus stopping protein synthesis. The occurrence of such glycoproteins in fungi has also been documented.[6,7] RIPs are three varieties: type 1 comprises a solitary polypeptide chainabout 28-33kDa showing N-glycosidase activity; type 2 consisting of two polypeptide chains which are connectedby a disulphide bond, of which one chain is comparable to the type 1 RIP and the second chain resembles lectinwhich facilitates the protein's penetration of the cell wall through binding to glycoreceptors on the cell surface. Type 3 RIPs are inactive proenzymes, which need activation to show catalytic activity.[8]

Endophytic fungi found in the hemiparasitic plant *Dendrophthoe falcata* on *Lagerstroemia speciosa* was used for extraction of these glycoproteins. There are only two reports available on endophytic fungi of *Dendrophthoe falcata*. Kumaresan and Suryanarayana[9] have reported several endophytic fungi from *Dendrophthoe falcata*. Govindappa *et al*.[7] and Govindappa *et al*.[10] have isolated the endophytic fungi *Cladosporium perangustum* and *Alternaria alternata* from *Dendrophthoe falcata*. Endophytic fungal RIPs exhibited potent anti-oxidant, anti-inflammatory, cytotoxic and anticancer properties.[6]

Aim of the research study is to isolate proteins from endophytic fungal species of parasitic plant *Dendrophthoe falcata* which grows on *Lagerstroemia speciosa* and examine the *In vitro* antioxidant and anti-inflammatory properties of endophytic fungal ribosome inactivating proteins.

MATERIALS AND METHODS

The hemiparasitic plant *Dendrophthoe falcata* grown on *Lagerstroemia speciosa* collected from Devarayanadurga region of Tumkur, Karnataka, India during January-March 2021 and September-December 2022 (in 2 different season dry and wetter). The plant was authenticated by Dr Haleshi C, Taxonomist, Davangere University, Botany Department, Davanagere, Karnataka, India and deposited in Botany department herbarium (Access No:HDUD 304). Leaves and stem samples were used for the studies. Samples collected in sterile polythene bags. The collected stem and leaves of plants was washed with running tap water and two times with sterilized distilled water to remove adhering materials and microbes.

Identification and isolation of endophytic fungal species

In aseptic conditions, the endophytic fungi was isolated and used standard procedure with respect to Sadananda *et al*.[11] The washed stem and leaves were surface sterilized with 0.1% $HgCl_2$ for 1 min followed by washing with distilled water 3-5 times. Leaves and stem were cut into small pieces of 0.5 to 1 cm using a sterilized surgical blade. Each stem and leaves segments was placed on Petri plates containing Potato Dextrose Agar (PDA) medium equidistantly. Plates were incubated at 30±2ºC for 8-10 days and allowed for growth of fungi and sporulation. The media was fortified with antibiotic chloramphenicol to prevent bacterial contamination. After 8-10 days of incubation, each individual fungal hyphae was selected and incubated again for 30±2ºC upto 1 week. Each individual isolates of fungi was numbered and transferred into PDA slants separately and maintained at 4ºC for further use.

The fungal species were identified according to their morphological characteristics: colony size, colour, structure and microscopic characteristics. The molecular identification was made by Eurofins® using ITS1 and ITS 4 primers. DNA is isolated from the culture. On a 1.0% Agarose Gel, its quality was assessed and a single band of high-molecular-weight DNA was seen.

PCR-amplified the portion of the ITS region and resolved on agarose, a single distinct PCR amplicon band of about 700 bp was seen. To get rid of impurities, the PCR amplicon was cleaned. ITS1 and ITS4 primers were used in the forward and reverse DNA sequencing reaction of the PCR amplicon, which was performed on an ABI 3730xl Genetic Analyzer using a BDT v3.1 cycle sequencing kit.

An alignment software-generated consensus sequence for the PCR amplicon based on forward and reverse sequence data. BLAST was performed using the ITS region sequence and the NCBI Genbank database. Clustal W, a multiple alignment software application, was used to pick and align the first 10 sequences based on the maximum identity score. MEGA 7 was used to create the distance matrix and phylogenetic tree. The detected fungi's sequences that have been added to NCBI Genbank.[12,13]

Mass production of obtained endophytic fungal species

The robustly growing fungus growing from the leaf identified and mass cultured in 250 mL Erlenmeyer flasks for extraction of proteins. Once sufficient quantity obtained, in 15 days, the mycelial mat was used for further work.

Isolation of crude Ribosome Inactivating Proteins and their Characterization

The crude extract was centrifuged (REMI-CPR 24 plus) at 10000 rpm and 4ºC for 30 min. The clear supernatant

measured and stored at -80ºC until further processing. This crude protein obtained after liquid nitrogen homogenisation was divided into two parts (50 mL). One part was used for Gel filtration chromatography and the other part used for affinity chromatography. SDS-PAGE gel (12%) was run to separate out the proteins. The gel filtration extract is used to test glycoproteins by staining with Periodic Acid Schiff (PAS) reagent staining method.

Molecular weight determination by SDS-PAGE

Polyacrylamide gel electrophoresis involved on thick vertical slab gel of 2 mm running 2 mm, utilizing 12.5% running gel and 5% stacking gels respectively. The dimensions of these gels that were cast were 10.2 cmx5 cmx0.75 mm. After dissolving the endophytic fungal RIPs in a 0.0625 M Tris-HCl (pH 6.8) solution with 1% SDS, 0.1% Coomassie Brilliant Blue (CBB) and 10% glycerol, the mixture was incubated for 5 min at 90ºC. The following molecular markers were utilized in the SDS-PAGE: myoglobin (29 kDa), ß-galactosidase (116 kDa), fructose-6-phosphate kinase (80 kDa), ovalbumin (68 kDa), glutamate (60 kDa), carbonic anhydrase (36 kDa) and bovine serum albumin (72 kDa) (Aristogene Bioscience Ltd., Bangalore). After loading the marker and RIPs samples into their corresponding wells, electrophoresis was carried out for 3 hr at a constant current of 50 V. With the completion of electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 and it was allowed to fix for 1 hr in a fixing solution. It was destained with a destaining solution. The molecular weight of the isolated RIPs was determined by electrophoretic mobility comparison with standard molecular weight marker proteins.

Periodic Acid Schiff reagent (PAS) staining for lectins/glycoproteins

Staining gels with Periodic Acid-Schiff (PAS) reagent is a confirmed method of detecting glycoproteins. The method used to detect the RIPs chain in ribosome inactivating proteins.[6,14,15] SDS-PAGE gel was soaked in 7.5% w/v acetic acid for 30 min at room temperature. It was then soaked in 0.2% periodic acid solution for 1 hr at 4ºC. The periodic acid was removed and Schiff reagent added without washing the gel. This incubated for 1 hr at 4ºC. After the removal of PAS reagent, the gel was left to soak in 7.5% acetic acid for 1 hr, then it was thoroughly cleaned and observed.

Hemagglutination assay

Two-fold serial dilution method was used for haemagglutination test performed in standard microlitre plates. Only five endophytic fungi were tested because the other two endophytes did not grow well in mass culture and were difficult to identify. An aliquot of 50 µL human erythrocyte (A+, B+, AB+, O+, B- and O-) solution was mixed with 50 µL of serially diluted protein and agglutination was evaluated following a 30 min incubation period at 25ºC. Hemagglutination activity is measured in units of the highest dilution of the protein that exhibited complete agglutination. Different sugars used to determine the sugar binding specificity of protein on N-acetylgalactose amine, D-galactose, D-mannose, D-glucose and maltose, which tested for their capability to prevent hemagglutination produced by proteins. Two-fold serial dilution of each carbohydrate in a range of 10 mM to 100 mM was prepared. The dilutions were then dissolved in a 0.15 M NaCl solution and combined with equivalent quantities of extract that exhibited four units. The mixtures were allowed to sit at room temperature for 30 min (25±2ºC), following a 4% human erythrocytes suspension was added and the entire sample was incubated for 1 hr. It was shown that the lowest concentration of carbohydrates completely inhibited hemagglutination.[6]

In vitro antioxidant activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) activity

DPPH is one of the widely used methods for identifying the radical scavenging activity of the compounds. Prepare a DPPH solution of 0.1 mM concentration in methanol. Dissolve the extracts of which scavenging activity is to be found in methanol in the increasing concentration. Later mix 1 mL of DPPH solution to the stock solution of extracts, incubate for 30 min in dark conditions. Later measured the absorbance at 517 nm using UV spectrophotometer. Methanol and ascorbic acid are used as blank and standard respectively. The Percentage of inhibition is calculated using the formula.

$$\text{Percentage of scavenging activity} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

FRAP (Ferric Reducing Antioxidant Power) activity

The FRAP method measures the reduction of ferric ions to ferrous ions which results in the development of coloured ions. Different concentrations of extracts are dissolved in distilled water. 600 microlitres of FRAP reagent was mixed with the endophytic fungal RIP's sample separately and kept for incubation in dark for 30 min. Then the antioxidant capacity can be measured spectrophotometrically at 593 nm.

$$\text{Relative percentage of reducing power} = \frac{\text{A–A min}}{\text{A–A min}} \times 100$$

In vitro anti-inflammatory studies

Albumin denaturation study

The standard anti-inflammatory study protocol used to analyze endophytic fungal RIPs for inhibition of albumin denaturation.[16] Study of albumin denaturation prevention or protein study was used to analyze anti-inflammatory potential and mechanism. All standard drugs are analyzed and approved based on this method of *In vitro* and *In vivo* studies. The drugs act as antigens and induce autoimmune diseases. The reaction mixture was made by using equal volume of varying concentrations of endophytic fungal RIPs (12.5 µg/mL to 100 µg/mL) and 1% aqueous bovine albumin. A small quantity of HCl used to change pH of the sample. Each sample mixture is incubated at 37ºC for 20 min then heated at 51ºC for another 20 min. At the end, percentage of inhibition of protein denaturation was calculated using following formula;

$$\text{Percent inhibition of albumin denaturation} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{efRIP}}}{\text{Abs}_{\text{control}}} \times 100$$

Where, efRIP-endophytic fungal RIP.

Study of anti-proteinase Activity

The anti-proteinase activity was studied for different concentrations of endophytic RIPs using the method of Sakat *et al*.[17] Proteinases play a vital role in causing inflammation. Drugs or natural products can hydrolyze peptide bonds and degrade inflammatory proteins. Atropine was used as a standard drug for comparison with other samples. The percent inhibition of proteases studied by using different concentration of endophytic fungal RIPs and calculated the activity by using below formula;

$$\text{Percentage of proteinase inhibition activity} = (1 - \frac{A_2}{A_1}) \times 100$$

Where, A1-absorbance of the control sample, Where, A2- absorbance of the efRIP.

Anti-Lipoxygenase activity study

Linoleic acid is used as a substrate and lipoxygenase is used as an enzyme in anti-lipoxygenase study.[18] The enzyme lipoxygenase pathway plays a vital role in the form of inflammation. The anti-inflammatory drugs inhibit the lipoxygenase activity through tissue regeneration enhancement.

RESULTS

Identification and isolation of endophytic fungi

Eight endophytic fungal species were isolated from stem and leaves of hemiparasitic plant *Dendropthoe falcata* that grew on *Lagerstroemia speciosa*. The fungi isolated from the leaf were *Diaporthe pterocarpi, Diaporthe pseudophoenicicola, Colletotrichum cobbittiense* and *Asteromella pistaciarum*. The fungi isolated from stem were *Colletotrichum siamense* and *Colletotrichum cobbittiense*.

Endophytic fungi identification was done based on morphological characters like colony size, colony structure and colour. Molecular level identification of fungi was done with the help of Eurofins, Bengaluru, Karnataka, India using ITS1 and ITS4 primers. The each endophytic fungal sequence were deposited in the NCBI Genbank and their accession numbers are PP159099, PP159096, OR858835 and OR827685 for future use of scientific community.

Mass production and extraction of Ribosome Inactivating Protein (RIP's) from endophytic fungal species

Many endophytic fungi were obtained from incubated parts of the plant parts and only a few of them were identified from molecular technique. All the endophytic fungal species were grown in Potato Dextrose Broth at 28±2ºC for 8-10 days for mass culture. Each endophytic fungal RIP was extracted from mycelial mat using 50 mL (1:25 w/v) of 154 mM NaCl in 50 mM sodium phosphate buffer (pH 7.2). Endophytic fungal RIP's was extracted from the mycelial mat of *Diaporthe pterocarpi, Asteromella pistaciarum*, *Diaporthe pseudophoenicicola*, *Colletotrichum siamense*, *Colletotrichum cobbittiense, Fusarium chlamydosporium, Alternaria* species. Each endophytic concentration found to be approximately 2.6 mg/mL.[6]

Molecular weight determination by SDS-PAGE

Results of SDS-PAGE showed all the endophytic fungal RIP's and the protein has a momolecular weight of protein was 68 kDa. Which confirms the low molecular weight of the proteins and it may be RIP. All the eight endophytic fungi *Diaporthepterocarpi, Diaporthe pseudophoenicicola, Colletotrichum cobbittiense, Asteromella pistaciarum*, *Colletotrichum siamense, Fusarium chlamydosporium* and *Alternaria* species showed the 68 kDa protein in the SDS-PAGE (Figure 1).

Determination of glycoprotein from PAS staining

All the endophytic fungal 68 kDa proteins showed purple-pink colour after the PAS staining. It confirms the 68kDa protein is glycoprotein. Subsequently, only endophytic fungi with molecular identities were

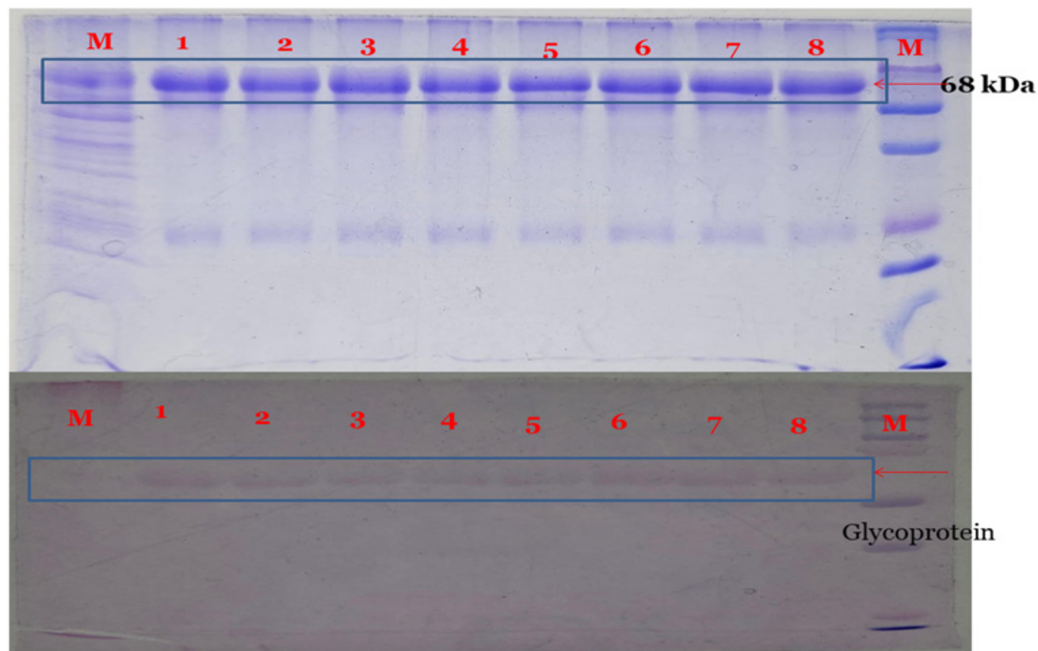

Figure 1: SDS-PAGE of different endophytic fungal RIPs and PAS staining showing all endophytes glycoproteins. *1-Diaporthe pterocarp, 2-Diaporthe pseudophenicola, 3-Colletotrichum cobbittiense, 4-Astromella pistachiarum, 5-Colletotrichum siamensis, 6-Fusarium chlamydosporium, 7-Phoma species and 8-Alternaria species.*

the subject of the same studies.[6] All the selected endophytic fungi expressed positive result for presence of glycoprotein from PAS staining by becoming purple-pink colour (Figure 1).

Hemagglutination assay

Hemagglutination assay was performed for the five endophytic fungal species RIP's using human erythrocytes and the data was represented in the Table 1. The DpRIP showed the positive result for the erythrocytes of A^+, B^+ and AB^+ and negative result for the B^- and O^- erythrocytes. The DpsRIP exhibited positive results for the B^+ and AB^+ erythrocytes and negative result for the other erythrocytes. The CcRIP expressed the positive result for the O^+ and B^- erythrocytes and negative result for the other erythrocytes. The ApRIP showed positive result for B^+ and AB^+ erythrocytes and negative result for others (Table 1).

Table 1: Hemagglutination assay for endophytic fungal species of *D. falcata*.

Blood group	Fungus 1	Fungus 2	Fungus 3	Fungus 4	Fungus 5
A+	+	-	-	-	-
B+	+	+	-	+	
O+		-	+	-	+
AB+	+	+	-	+	-
B-	-	-	+	-	+
O-	-	-		-	-

+ = indicates presence, - = indicates absence.
Fungus 1- *Diaporthe pterocarpi*, Fungus 2- *Diaporthe pseudophoenicicola*, Fungus 3- *Colletotrichum cobbittiense*, Fungus 4- *Asteromella pistaciarum*, Fungus 5- *Colletotrichum siamense*

Antioxidant activity

DPPH assay

The DPPH assay was conducted to evaluate the antioxidant capacity of RIPs extract of fungi, *Diaporthe pterocarpi, Diaporthe pseudophoenicicola, Colletotrichum cobbittiense, Asteromella pistaciarum*, *Colletotrichum siamense*. Figure 2 depicts the results of antioxidant activity of RIPs. The 100 μg extract of CcRIP showed the highest antioxidant scavenging activity (78.41 μg/mL) followed by DpsRIP (76.82 μg/mL), ApRIP (71.89 μg/mL), CsRIP (68.94 μg/mL) and DpRIP (64.63 μg/mL) respectively. The scavenging activity was dependent on dose concentration and was compared with the standard drug Ascorbic acid 100 μg/mL.

FRAP assay

FRAP assay used to determine the antioxidant capacity of extracts of endophytic fungi, *Diaporthe pterocarpi, Diaporthe pseudophoenicicola, Colletotrichum cobbittiense, Asteromella pistaciarum*, *Colletotrichum siamense*. Figure 3 depicts the results of FRAP antioxidant activity of RIPs. DpsRIP and CcRIP showed significant scavenging activity (71.83 μg/mL and 73.49 μg/mL). ApRIP (64.49 μg/mL),

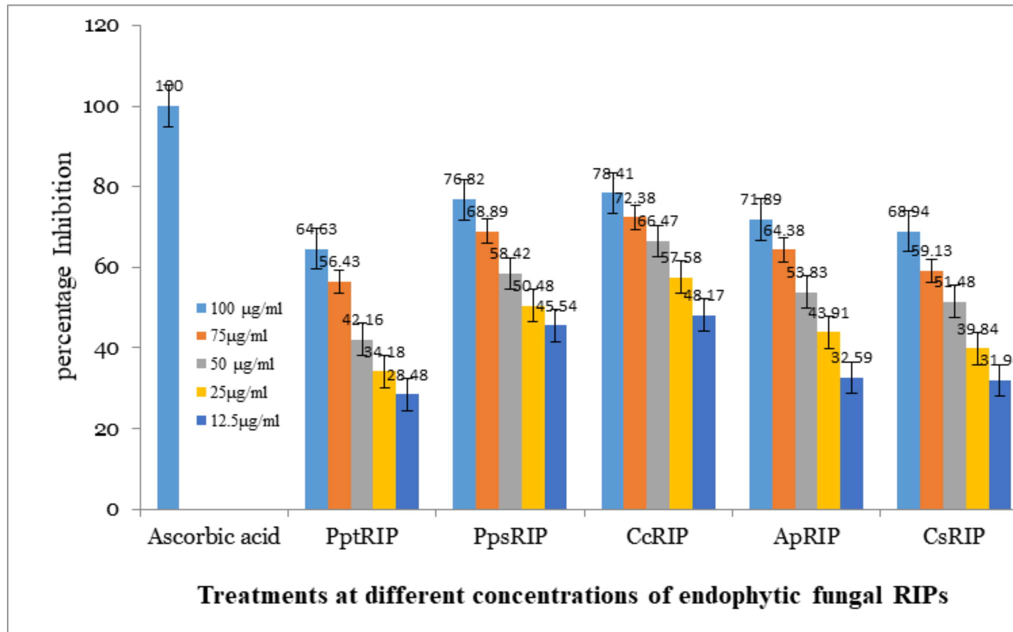

Figure 2: *In vitro* DPPH scavenging activity of endophytic fungal RIPs.

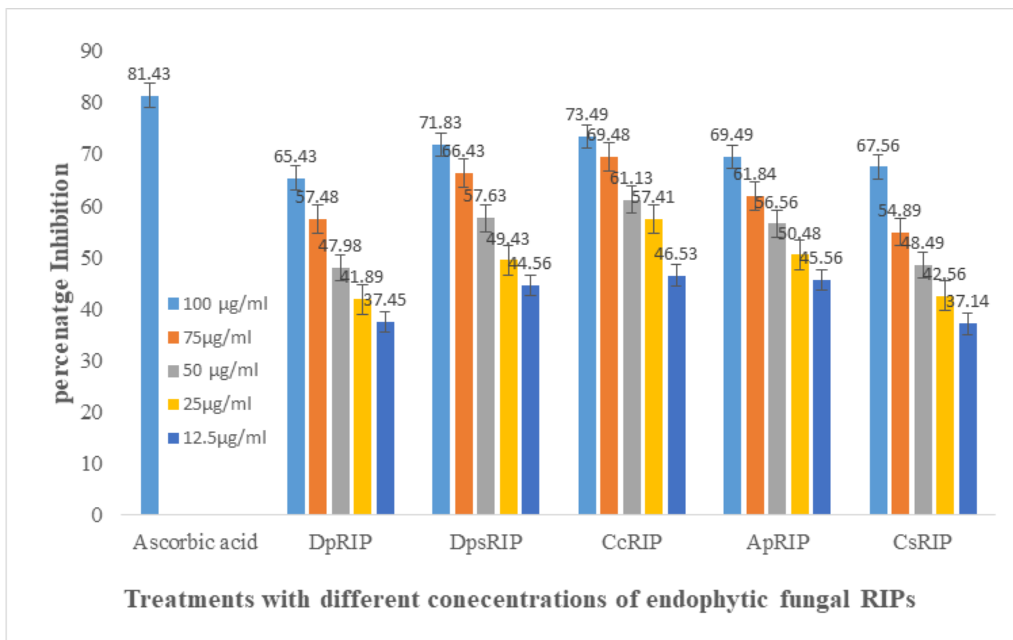

Figure 3: *In vitro* FRAP Scavenging activity of endophytic fungal RIPS.

CsRIP (67.56 µg/mL) and DpRIP (65.43 µg/mL) showed moderate activity in the decreasing order.

In vitro anti-inflammatory properties on endophytic fungal RIPs

Albumin denaturation inhibition study

The five different endophytic fungal RIPs of various concentrations ranging from 12.5 to 100 µg/mL were used for albumin denaturation inhibition study. The obtained results were compared with standard drug, atropine. The CcRIP significantly inhibited the albumin denaturation by 89.71 at 100 µg/mL followed by PpsRIP (85.18), CsRIP (83.41), ApRIP (82.19) and PptRIP (80.92) and the activity was dose-dependent. Whereas the standard drug showed an activity of 101.42 at 100 µg/mL (Figure 4).

Study of anti-proteinase efficacy

All five endophytic fungal RIPs at different concentration were tested for proteinase inhibitory efficacy and the CcRIP (98.48±0.12) were found to exhibit strong proteinase inhibitory activity followed

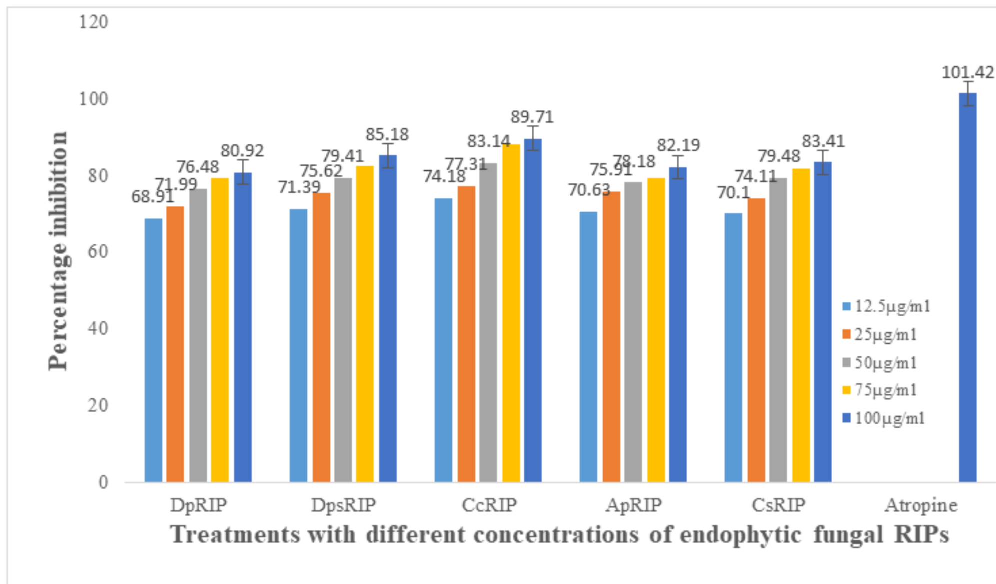

Figure 4: Effects of endophytic fungal RIPs on albumin denaturation inhibition.

by PpsRIP (94.42±0.14), ApRIP (91.08±0.15), CsRIP (89.35±0.15) and PptRIP (87.41±0.14) at 100 µg/mL and the activity increased when the concentration was increased. Atropine showed 107.68±0.04 at 100 µg/mL (Figure 5).

Anti-lipoxygenase activity study

The maximum anti-lipoxygenase activity was observed in CcRIP and was concentration dependent manner. The highest activity among all endophytic fungal RIPs observed (96.68, 89.74, 87.77, 84.78, 78.33) at 100 µg/mL compared with standard atropine (101.46) (Figure 6).

DISCUSSION

All (08) the endophytic had exhibited the 68 kDa protein in SDS-PAGE. For further studies, only 5 endophytic fungi were selected from *Dendrophthoe falcata* namely *Diaporthe pterocarpi, Diaporthe pseudophenicola, Colletotrichum cobbittiense*, *Astromella pistachiarum* and *Colletotrichum siamensis* based on molecular identification. These five endophytic fungal RIPs were examined the anti-oxidant and anti-inflammatory properties. These results provided insights into the biological activities of these fungi, which have significant implications in various applications including medicine and agriculture. Our studies are in confirmations with previous studies

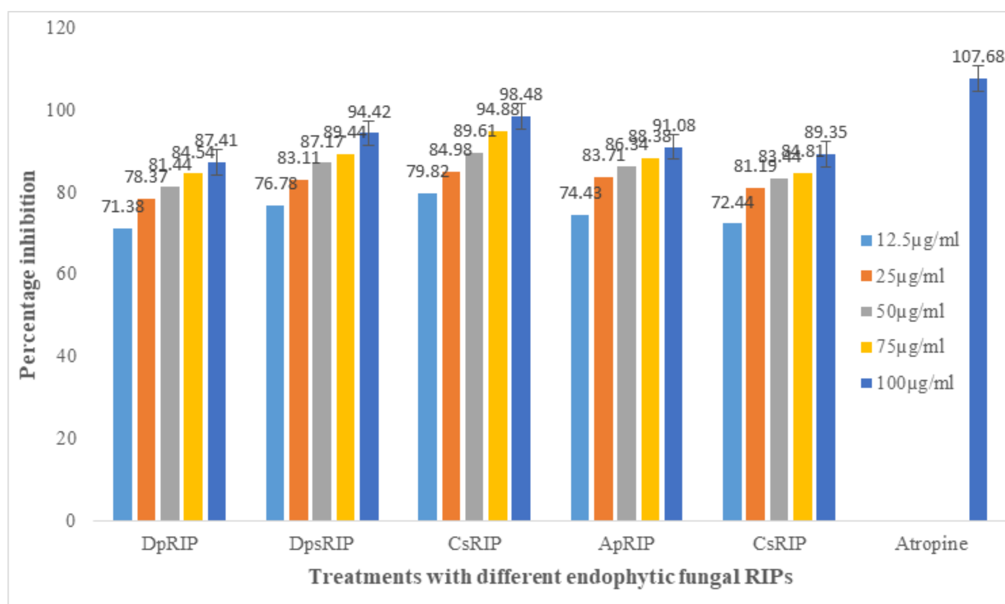

Figure 5: Effects of endophytic fungal RIPs on anti-proteinase activity.

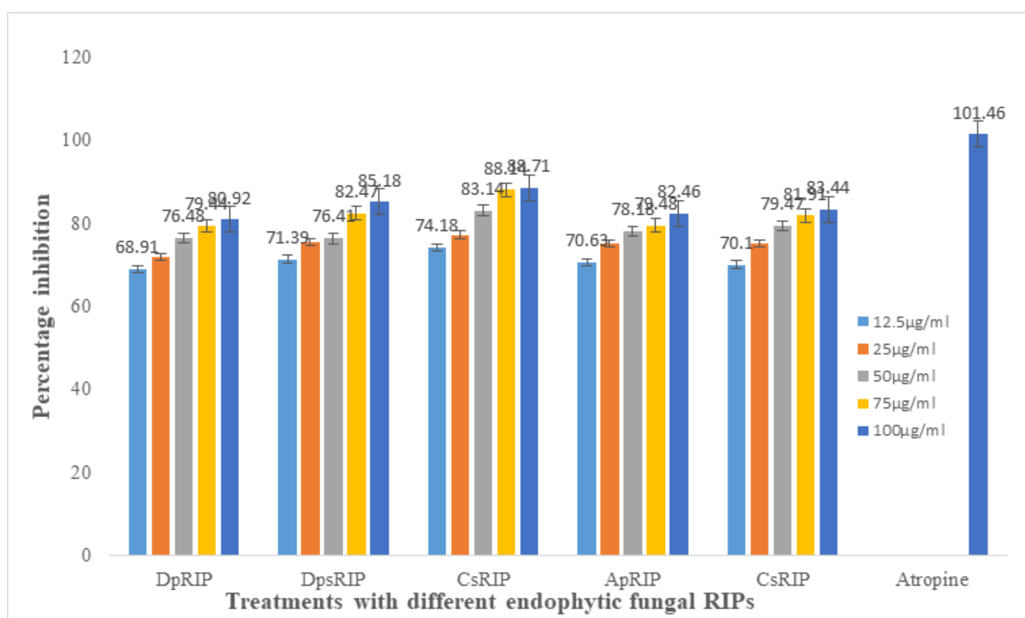

Figure 6: Anti-lipoxygenase activity of endophytic fungal RIPs.

including Sadananda *et al*.[6] and Cheuthong *et al*.[14] who have reported similar findings in endophytic fungi. The identification of Diaporthe pterocarpi and its ribosome inactivating properties is particularly noteworthy. Our findings ings align with those of Sadananda *et al*.[6] who reported endophytic fungi with hemagglutination properties in erythrocytes of different blood types. This similarity suggests a potential common pathway or mechanism that could be studied further. The CcRIP expressed the strong positive result for the O+ and B- erythrocytes and negative result for the other erythrocytes. The ApRIP showed positive result for B+ and AB+ erythrocytes and negative results for others.[19-21]

The strong antioxidant activities observed in CcRIP and DpRIP, as confirmed by both the DPPH and FRAP assays, indicate that these endophytic fungi could be potent sources of natural antioxidants. This is consistent with the findings of Gholizadeh,[22] who reported significant antioxidant activities in in similar fungi. These results suggest that CcRIP and DpRIP may contribute to antioxidant mechanisms, potentiality offering benefits in medical or agricultural applications. Recent studies by Hashem *et al*.[23] and Kousar *et al*.[24] have highlighted the importance of endophytic fungi in producing bioactive compounds. Our studies have reinforced these findings and supplement the existing literature on potential applications of these fungi. The present study, the CcRIP and DpRIP exhibited strong antioxidant activity in DPPH method. The RIPs may contribute significantly responsible for antioxidant activity. The results are significant and the results confirmed with the findings of Gholizadeh (2019),[22] Ali *et al*. (2016),[25] Ilahi *et al*. (2013).[26] In FRAP assay, the CcRIP and DpRIP strongly exhibited antioxidant activity in FRAP method. The results are confirmatory with the findings Gholizadeh (2019),[22] Ali *et al*. (2016),[25] Ilahi *et al*. (2023).[26]

In anti-inflammatory activity, inhibition of albumin denaturation was noticed from CcRIP followed by DpRIP, CsRIP, DpsRIP and ApRIP. The entire endophytic fungal RIP inhibited the denaturation of albumin. The significant anti-proteinase property was observed from all the endophytic fungal RIPs and it was depending on concentration dependent. The CcRIP significantly inhibited the albumin denaturation (89.71) at 100 µg/mL followed by PpsRIP (85.18), CsRIP (83.41), ApRIP (82.19) and PptRIP (80.92) and the activity was dose dependent. Whereas the standard drug showed 101.42 at 100 ug/mL. Plants (castor),[27,28] *Trichosanthes cucumerina,*[29] elderberry,[30] momordica[31,32] RIPs expressed potent anti-inflammatory activity.

Plants RIPs reported as potent anti-proteinase activity and maximum The maximum anti-lipoxygenase activity was observed from CcRIP and it was concentration dependent. The highest activity was noticed at high concentration of all the endophytic fungal RIPs. The (castor),[27,28] *Trichosanthes cucumerina,*[29] elderberry,[30] Momordica[31,32] RIPs expressed potent anti-inflammatory activity.

SUMMARY AND CONCLUSION

For the first time, we reveal the anti-inflammatory and antioxidant properties of endophytic fungal crude RIPs from *Dendrophthoe falcata*. The CcRIP and ApRIP has significant anti-inflammatory and anti-inflammatory activity. This activity is based on RIPs concentration. Endophytic fungal RIPs are glycoproteins and showed hemagglutination property. These RIPs can be utilised as a source of natural antioxidant and anti-inflammatory agents. Further, these RIPs can be characterized by LC-MS/MS and MALDI-TOF-MS to identify and characterize and *In vitro* studies will recommend our data.

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CONFLICT OF INTERESTS

All the authors declare no conflict of interest.

ABBREVIATIONS

RIP: Ribosome Inactivating Proteins; **DpRIP:** *Diaporthe pterocarpi* Ribosome Inactivating Proteins; **DpsRIP:** *Diaporthe pseudophoenicicola* Ribosome Inactivating Proteins; **CcRIP:** *Colletotrichum cobbittiense,* Ribosome Inactivating Proteins; **ApRIP:** *Asteromella pistaciarum* Ribosome Inactivating Proteins; **CsRIP:** *Colletotrichum siamense* Ribosome Inactivating Proteins.

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