IJPSR (2021), Volume 12, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 August 2020; received in revised form, 09 January 2021; accepted, 07 April 2021; published 01 August 2021

IN-VITRO BIOACTIVE POTENTIAL OF ENDOPHYTIC FUNGI *CURVULARIA LUNATA* ISOLATED FROM MEDICINAL PLANT *FICUS RELIGIOSA*

K. K. Jayant * and B. S. Vijayakumar

Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam, Puttaparthi, Anantapur - 515134, Andhra Pradesh, India.

Keywords:

Endophytic fungi, *Curvularia lunata*, anti-oxidant, anti-diabetic, anti-inflammatory

Correspondence to Author:
K. K. Jayant

Doctoral Research Scholar,
Department of Biosciences,
Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam,
Puttaparthi, Anantapur - 515134,
Andhra Pradesh, India.

E-mail: kottapallikjayant@sssihl.edu.in

ABSTRACT: Several studies undertaken in the recent past have demonstrated that endophytic fungi are capable of producing a number of important bioactive secondary metabolites, hitherto known only from plants, thereby raising the potential of using endophytes as alternative sources for these metabolites. Occasionally, the compounds produced by endophytic fungi are similar to those produced by the respective host plants. The plant chosen for the current study has significant medicinal and therapeutic value, and endophytic fungi were isolated from the leaves of this plant. The leaves have been specifically chosen for this study as there is significant evidence suggesting that the leaf extracts of these plants have higher medicinal value. The hypothesis of this current study is that endophytic fungi isolated from leaves of the medicinal plant and its extract would exhibit similar or greater medicinal properties as that of leaf extracts. This work intends to highlight the bioactive potential of endophytic fungus *Curvularia lunata* isolated from *Ficus religiosa*. Extracts obtained from the fungi understudy were tested *in-vitro* for their possible applications as anti-oxidant, anti-diabetic and anti-inflammatory agents.

INTRODUCTION: Natural products are naturally derived metabolites and/or byproducts from microorganisms, plants, or animals. These products have been exploited for human use for thousands of years. Plants have been the chief source of such compounds used in medicine. Although plants are the major source of modern drugs, there is a continuous search for newer sources to obtain new lead molecules, with higher biological properties, for treating various diseases. De Bary in 1866 [1], first introduced the term 'endophyte'. Interestingly, every plant is the host for one or more endophytic microorganisms.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(8).4322-30

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(8).4322-30

Endophytes are the microorganisms that grow and colonize inside the internal living tissues of the host plant without causing any negative effect to it [2]. Endophytes include both fungi and bacteria, but the former is ubiquitous, versatile, and widespread microorganisms, which colonize on plants and grow in almost all geo-climatic conditions. They conquer the internal plant tissues below the epidermal cell layers without causing any probable harm or symptomatic infection to their host and live within the intercellular spaces of the tissues [3].

Endophytic fungi arean alternate and relatively new source of pharmacologically active secondary metabolites, and over the past few decades, it has gained importance and relevance based on the understanding that they exist in a symbiotic relationship within their plant host. Fungal endophytes are known to produce an array of bioactive molecules with diverse activities such as

antimicrobial, antioxidant, cytotoxic, immuno-suppressive, and anti-inflammatory activities.

In this study, *in-vitro* anti-oxidant, anti-diabetic and anti-inflammatory potential of endophytic fungus *Curvularia lunata* was analyzed by biochemical assays along with phytochemical analysis and quantification of major secondary metabolites produced by the fungus.

MATERIALS AND METHODS:

Collection of Plant Samples and Culturing Endophytic Fungus: Fresh and healthy leaf samples of *Ficus religiosa* (plant specimen identified and authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore) were collected from various areas of Puttaparthi, the place of the current study. The plant specimens were processed by thoroughly rinsing the leaves of selected plants with tap water. Samples were cut into 3-4 cm pieces prior to rigorous surface sterilization (75% Ethanol for 2-5 minutes, followed by 4% Sodium hypochlorite for 1-2 minutes and again 75% Ethanol for 2-5 minutes). Surface sterilized tissues were rinsed three times with sterile water, blot dried, cut into small pieces (1–1.5 cm), and plated on Potato Dextrose Agar (Hi-Media Laboratories) plates supplemented with Streptomycin (250 mgL^{-1}). The efficacy of surface sterilization was confirmed by inoculating the surface-sterilized water collected from the last wash of the sample in a nutrient medium. The absence of growth of any fungi on the media confirms the efficient surface sterilization of the segments. The inoculated petri plates were sealed and incubated at 25°C in an incubation chamber. After 3 days of inoculation, the plates were observed daily for the growth of the fungi from cultured segments up to two weeks. The fungus that emerged from the tip of the segment was picked up, sub-cultured, and the pure culture was maintained for the extraction of fungal metabolites.

Phenotypic and Genotypic Identification of the Endophytic Fungus: The isolated endophytic fungus was initially identified through microscopic examination of colony morphology and reproductive characteristics using slide cultures. Primary confirmation of morphological identification of the endophytic fungi was carried out at ITCC, Division of Plant Pathology, Indian Agricultural Research Institute, Delhi. For genotypic identification, total genomic DNA of the endophytic fungi was isolated directly from actively growing mycelium growing in potato dextrose broth (PDB), using a DNA extraction kit (Genei) [4]. The extracted DNA was subjected to the polymerase chain reaction (PCR) using primers ITS1: 5'-CGGGATCCGTGG TGAACCTG- 3' and ITS4: 5'-CGGGTCCTCCG CTTATTGATATGC- 3' for amplification of the ITS region. Amplified DNA was subjected to DNA sequencing, and this DNA sequence was compared with already existing DNA sequences in NCBI GenBank (http://www.ncbi.nlm.nih.gov.blast) to identify the respective fungi. PCR and DNA sequencing was done by the Pentavalent Biotechnology Pvt., Ltd, Bengaluru. The acquired gene sequence was submitted to the NCBI Gen Bank database, and an accession number was obtained (MT825246).

Extraction of Fungal Metabolites: The mycelia of *Curvularia lunata*was inoculated to Potato Dextrose broth (PDB) for production of fungal metabolite by fermentation method. The mycelia of *C. lunata* from growing edge was inoculated into 1000 mL Erlenmeyer flask containing 500 mL of PDB medium and kept on a rotary shaker for 14-21 days at 25 °C. The culture filtrate was filtered using Whatman filter paper. The filtrate was extracted with the same volume of ethyl acetate twice and the extract was concentrated using a rotary evaporator. Then it was extracted successively with different organic solvents according to polarity range such as chloroform, dimethyl suphoxide (DMSO) and methanol to obtain chloroform, DMSO and methanol fractions [5]. These crude extracts were evaporated using a rotary evaporator under reduced pressure to obtain concentrated extracts.

Qualitative Phytochemical Analysis: The endophytic fungal extracts were tested for the presence of the following secondary metabolites by various qualitative techniques [6, 7].

Alkaloids: The endophytic fungal crude extract was evaporated, and the residues were dissolved in 2N HCl. The mixture was filtered, and the filtrate was used for the following test:

Wagner's Test: Reddish-brown precipitate formation indicates presence of alkaloids.

Hager's Test: Presence of alkaloid was confirmed with yellow colour precipitate.

Flavonoids:

Alkaline Reagent Test: Fungal extract was treated with 10% ammonium hydroxide solution. Presence of flavonoids was detected by a yellow fluorescence.

Shinoda Test: To 0.5 ml of crude extract, 5–10 drops of dil. HCl and a small piece of zinc was added followed by a boiling solution for few minutes. In the presence of flavonoids, pink or dirty brown colour was produced.

Phenol:

Ferric Chloride Test: Extract treated with 5% ferric chloride solution. The dark green colour indicates the presence of phenol.

Lead Acetate Test: 10% lead acetate solution was treated with fungal crude extract, and a bulky white colour indicates phenolic presence.

Steroid:

Salkowski Test: To 2 ml of chloroform, 0.5 ml of extract was added and mixed. Sulphuric acid was added along walls and allowed to stand for some time after shaking. A reddish-brown colour at the interface indicates the presence of the steroidal ring.

Terpenoids:

The Libermann-Burchard Test: A deep red layer at the junction of 2 layers indicates the presence of terpenoids when 1 ml of the fungal crude extract was treated with few drops of acetic acid and 1ml concentrated sulphuric.

Quantitative Phytochemical Analysis:

Total Flavonoid Content: Total flavonoid content was estimated by a colorimetric method [8]. Briefly, 0.5 ml of 2% ethanolic $AlCl_3$ solution was added to 0.5 ml of endophytic fungal crude extract. After 1 h the absorbance was measured with UV-Spectrophotometer (Spectramax M5, Molecular Devices) against the blank at 420 nm. The yellow colour indicated the presence of flavonoids. Extracted samples were evaluated at a final concentration of (1 mg/ml).

All the tests were carried out in triplicates, and the results were expressed as Quercetin equivalent (µg of Quercetin per mg of extract).

Determination of Total Phenolic Content: Total phenol content of extracts of *C.lunata* was estimated using Folin-Ciocalteau reagent-based assay using gallic acid as standard [9]. To the extract (1 mg/mL), 500µL of (50%) Folin-Ciocalteau reagent was added, followed by the addition of 1.5 mL of 20% of Na_2CO_3. The final volume was made up to 5 mL by adding distilled water. The mixture was incubated at room temperature for 30 min, and the absorbance of the developed colour was recorded at 765 nm using UV-Vis spectrophotometer. The same procedure was repeated with 1 mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions used as a standard for the calibration curve. Total phenolic value was obtained from the regression equation: $y = 0.003x+0.062$ with $R^2 = 0.999$ and expressed as mg/g gallic acid equivalent using the formula: C = cV/M, where C is the total content of phenolic compounds in mg/g GAE; c is the concentration of gallic acid established from the calibration curve; V is the volume of extract and M is the weight of the fungal extract.

Anti-oxidant Activity of *C.lunata:*

DPPH Free Radical Scavenging Assay: DPPH [2,4,6-$(O_2N)_3C_6H_2NHN(C_6H_5)_2$] is a stable, nitrogen-centered free radical which produces violet colour in ethanol/methanol solution. Change in absorbance of DPPH was monitored for anti-oxidant activity through free radical scavenging. Endophytic fungal extracts at 1mg/ml concentration were used. In the presence of a substrate that can donate hydrogen atom added in DPPH solution, it is reduced to yellow coloured product, diphenyl-picryl hydrazine. DPPH solution (0.5 mM/L) was prepared in 95% methanol, and a total of 2ml of DPPH solution was added to the test sample and incubated for 30 min at room temperature in darkness. After incubation, the absorbance was measured at 517 nm [10]. Ascorbic acid was taken as standard. Free radical scavenging activity was expressed as percentage inhibition, calculated as:

$$\text{Inhibition (\%)} = A_{\text{control}} - A_{\text{sample}} \times 100 / A_{\text{control}}$$

Hydrogen Peroxide Scavenging (H_2O_2) Assay: The ability of fungal extracts to scavenge hydrogen peroxide was estimated. Solution of hydrogen peroxide (40mM/L) was prepared in phosphate buffer (50 mM/L; pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Endophytic

fungal extracts (1mg/ml) were added to H_2O_2, and absorbance recorded at 230 nm was determined after 10 min, against a blank solution containing phosphate buffer without H_2O_2 [11].

H_2O_2 scavenging activity calculated and depicted as a percentage:

$$\text{Scavenged } H_2O_2\,(\%) = A_{\text{control}} - A_{\text{sample}} \times 100 / A_{\text{control}}$$

Inhibition of Carbohydrate Hydrolysing Enzymes (α-amylase and α- glucosidase) (Anti-diabetic Activity):

Alpha Glucosidase Inhibition (AGI) Assay: The AGI assay was performed using ρ-nitro phenyl-α-D-glucopyranoside as a substrate. The reaction mixture was prepared by adding 50 µl of phosphate buffer (50 mM, pH-6.8), 10 µl of an α-glucosidase enzyme from *Saccharomyces* sp. (1U/ml), and 20 µl of test sample followed by incubation for 5 min at 37 °C. After incubation, 20 µl substrate was added and incubated for 30 min at 37 °C. The reaction was terminated by adding 50 µl of sodium carbonate[12]. All the reactions were performed in triplicates in a 96-well microtiter plate. Absorbance was taken at 405 nm, and activity was calculated using the following formula:

$$\%\ \text{Alpha-glucosidase Inhibition} = A_{\text{control}} - A_{\text{sample}} \times 100 / A_{\text{control}}$$

Where, control is the solution having all reagents except the test sample. Acarbose was used as a standard drug and compared with the test samples.

Alpha-Amylase Inhibition (AAI) Assay: Reaction mixture was prepared by adding 40 µl of fungal extract, 200 µl of phosphate buffer (pH 6.9), and 40 µl (24 U/ml) porcine pancreatic amylase. It was incubated at 37 °C for 10 min, followed by addition of 50 µl of starch (1%) and incubation at 37°C for 20 min. The reaction was terminated by adding 0.5 ml DNS reagent followed by incubation in boiling water for 5 min. The reaction mixture was diluted with 5 ml of distilled water, and absorbance was read at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 40 µl of a buffer. Control representing enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol[13].

The AAI activity was calculated using the formula:

$$\%\ \text{Alpha-amylase Inhibition} = A_{\text{control}} - A_{\text{sample}} \times 100 / A_{\text{control}}$$

Where, control is the solution having all reagents except the test sample. Acarbose was used as a standard drug and compared with the test samples.

Determination of Glucose Uptake Capacity by Yeast Cells: Commercial baker's yeast was dissolved in distilled water to prepare 1% suspension. The suspension was kept overnight at room temperature. The next day, yeast cells suspension was centrifuged at 4200 rpm for 5 min. The process was repeated by the addition of distilled water to the pellet until a clear supernatant was obtained. A 10% *v/v* suspension of the yeast cells solution was made for the assay.

Different concentrations of Ethyl acetate extract (1-5 mg/ml) were taken. The mixture was then supplemented with various concentrations (5, 10, and 25 mM) of 1 mL of glucose solution and incubated for 10 min at 37 °C. To initiate the reaction, 100 µl of yeast suspension was poured in the mixture of glucose and extract, vortexed, and incubated for another 60 min at 37 °C[14].

After incubation, the tubes were centrifuged for 5 min at 3800 rpm, and glucose was estimated by using a spectrophotometer at 520 nm. Absorbance for the respective control was also recorded on the same wavelength. The percentage increase in uptake was calculated by the formula:

$$\%\ \text{increase in glucose uptake} = A_{\text{control}} - A_{\text{sample}} \times 100 / A_{\text{control}}$$

Where control is the solution having all reagents except the test sample. Metronidazole was used as standard drug and compared with the test samples.

Anti-inflammatory Activity:

Inhibition of 15-lipoxygenase (15-LOX) Enzyme: Inhibition of Soya bean 15-LOX was determined in this assay [15]. The substrate 0.2µM linoleic acid was prepared in 0.2M borate buffer (pH 9). Different concentrations of endophyte extracts were mixed with 15-LOX enzyme and incubated for 5 min at room temperature. The substrate was added to the mixture, and the absorbance was measured at 243 nm using a UV-Vis spectrophotometer. Quercetin was used as positive control and methanol as a negative control.

% Inhibition of 15-LOX was calculated as:

$$\%\ \text{Inhibition} = (OD_{\text{extract}} - OD_{\text{blank}}) \times 100\ (OD_{\text{negative control}} - OD_{\text{blank}})$$

RESULTS:

Morphological Identification of *Curvularia lunata*: Colonies appeared brown with a black reverse. Conidiophores erect and nodulose. Conidia are ellipsoidal, curved or lunate, rounded at the ends or sometimes tapering slightly towards the base, pale brown, medium reddish-brown to dark brown [16].

FIG. 1 & 2: *CURVULARIA LUNATA*, 40 X MAGNIFICATION OF *C. LUNATA* GROWING ON PDA

Qualitative Analysis: Screening of various phytochemicals of Ethyl acetate extracts reveals a good concentration of alkaloids, phenols, flavonoids, tannins, and sterols in endophytic fungi *C. lunata*, as shown in **Table 1**. These chemical compounds are responsible for different medicinal properties of extracts.

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ETHYL ACETATE EXTRACTS

Test	Alkaloids	Flavonoids	Phenol	Steroids	Terpenoids
Wagner's Test	√				
Hager's Test	√				
Alkaline reagent Test		√			
Shinoda Test		√			
Ferric Chloride Test			√		
Lead Acetate Test			√		
Salkowski Test				√	
Libermann-Bruchard Test					√

Determination of Total Flavonoid Content: There was a range of flavonoid concentrations in different organic fractions of *C. lunata* extracts. The highest concentration of flavonoid was observed in Ethyl acetate extract with a concentration of 1.98 mg µg Quercetin/mg.

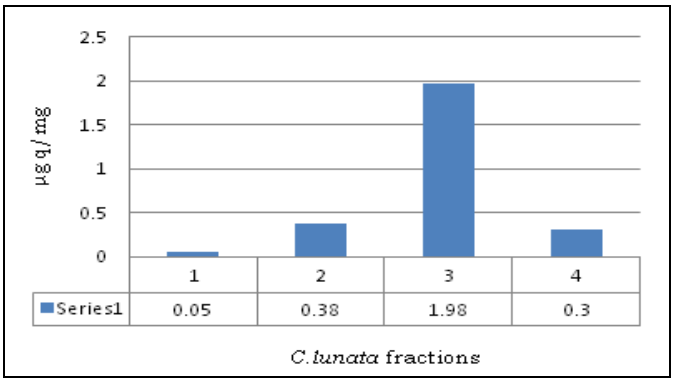

FIG. 3: TOTAL FLAVONOID CONTENT. Legend: 1-Methanol fraction, 2- Chloroform fraction, 3- Ethyl acetate fraction, 4-DMSO fraction

Determination of Total Phenol Content: There was a range of phenolic concentrations in different organic fractions of *C. lunata* extracts. The highest concentration of phenol was observed in Ethyl acetate extract with a concentration of 19.83 mg GAE/g of dry gallic acid.

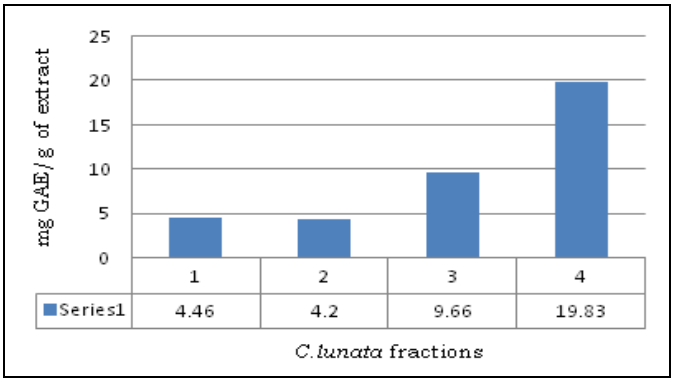

FIG. 4: TOTAL PHENOL CONTENT. Legend: 1-Methanol fraction, 2- Chloroform fraction, 3- DMSO fraction, 4-Ethyl acetate fraction

Anti-oxidant Activity: In the present study; Chloroform, Dimethyl suphoxide, Ethyl acetate, and Methanol fractions of *C. lunata* extracts were tested using two different methods. All fractions showed considerable activity with varying extent. Fungal extracts having anti-oxidant potential also

had a good amount of total phenols as tested by Folin-Ciocalteau reagent based assay

DPPH Free Radical Scavenging Assay: The reaction was visible as a colour change from purple to yellow. Ethyl acetate fraction showed high anti-oxidant capacity value of 71.25%. Ascorbic acid as standard showed 90% inhibition activity **Fig. 5**. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Though, the DPPH radical scavenging potential of the extract was less than that of ascorbic acid; the study however showed that the extract has an average proton-donating ability and could be a potential free radical inhibitor.

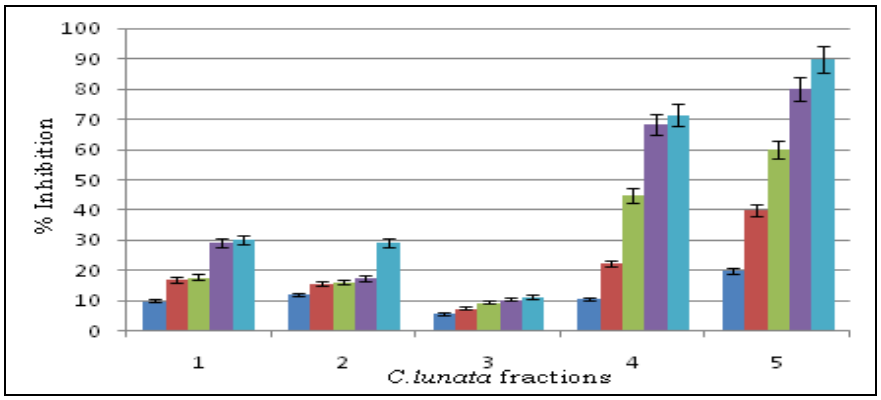

FIG. 5: DPPH SCAVENGING ACTIVITY. 1. Methanol fraction - (1.1 - 2mg/ml, 1.2 - 5 mg/ml, 1.3 – 10 mg/ml, 1.4 – 15mg/ml, 1.5 – 20mg/ml). 2. Chloroform fraction - (2.1 - 2mg/ml, 2.2 - 5 mg/ml, 2.3 – 10 mg/ml, 2.4 – 15mg/ml, 2.5 – 20mg/ml). 3. DMSO fraction - (3.1 - 2mg/ml, 3.2 - 5 mg/ml, 3.3 – 10 mg/ml, 3.4 – 15mg/ml, 3.5 – 20mg/ml). 4. Ethyl acetate fraction - (4.1 - 2mg/ml, 4.2 - 5 mg/ml, 4.3 – 10 mg/ml, 4.4 – 15mg/ml, 4.5 – 20mg/ml). 5. Standard - Ascorbic acid (5.1 - 2mg/ml, 5.2 - 5 mg/ml, 5.3 – 10 mg/ml, 5.4 – 15mg/ml, 5.5 – 20mg/ml)

Hydrogen Peroxide Scavenging (H_2O_2) Assay: As shown in figure, the reduction potential of fungal extracts in (H_2O_2) assay ranged from 8% to 63.5% over various extracts and concentrations. Ethyl acetate extract showed comparable results with that of standard ascorbic acid (90%) **Fig. 6**.

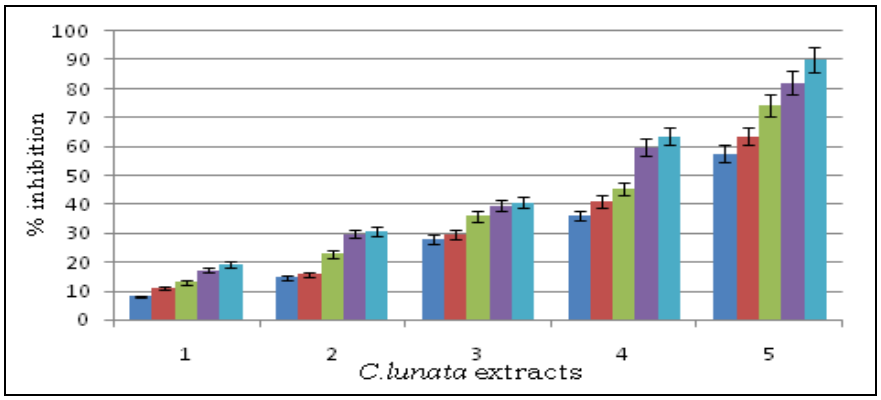

FIG. 6: HYDROGEN PEROXIDE SCAVENGING ACTIVITY. 1. DMSO fraction (1.1 - 2mg/ml, 1.2 - 5 mg/ml, 1.3 – 10 mg/ml, 1.4 – 15mg/ml, 1.5 – 20mg/ml. 2. Methanol fraction - (2.1 - 2mg/ml, 2.2 - 5 mg/ml, 2.3 – 10 mg/ml, 2.4 – 15mg/ml, 2.5 – 20mg/ml . 3. Chloroform fraction- (3.1 - 2mg/ml, 3.2 - 5 mg/ml, 3.3 – 10 mg/ml, 3.4 – 15mg/ml, 3.5 – 20mg/ml . 4. Ethyl acetate fraction - (4.1 - 2mg/ml, 4.2 - 5 mg/ml, 4.3 – 10 mg/ml, 4.4 – 15mg/ml, 4.5 – 20mg/ml. 5. Standard - Ascorbic acid - (5.1 - 2mg/ml, 5.2 - 5 mg/ml, 5.3 – 10 mg/ml, 5.4 – 15mg/ml, 5.5 – 20mg/ml

Anti-diabetic Activity:

Alpha Glucosidase Inhibition Assay: Antidiabetic activity of various fungal extracts was determined by inhibition of α-amylase. All the fractions showed considerable activity **Fig. 7**, the Ethyl acetate extract at maximum concentration exhibited inhibition of 62.86%. α-glucosidase inhibition of standard drug Acarbose was 99.24%.

α-amylase Inhibitory (AAI) Assay: The result of inhibition of α-amylase of the various fungal extract showed the range from 9.04% to 56.76% **Fig. 8**.

The ethyl acetate extract showed maximum inhibition of 56.76%, while standard Acarbose exhibited 75.37% inhibition of the enzyme.

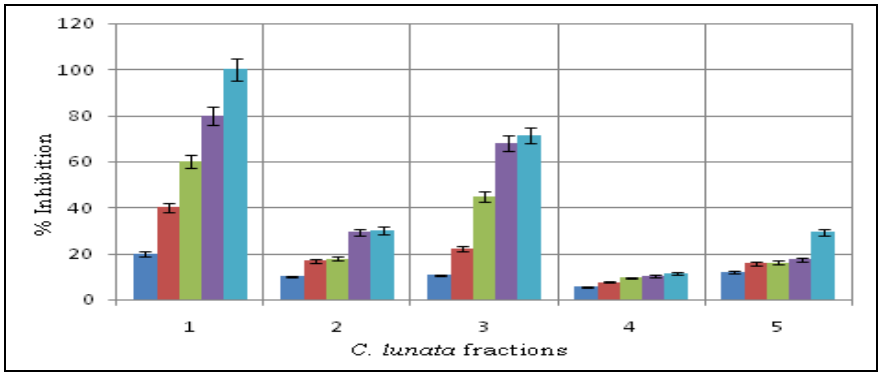

FIG. 7: ALPHA GLUCOSIDASE INHIBITION. 1. Standard - Acarbose (1.1 - 2mg/ml, 1.2 - 5 mg/ml, 1.3 – 10 mg/ml, 1.4 – 15mg/ml, 1.5 – 20mg/ml. 2. Methanol fraction - (2.1 - 2mg/ml, 2.2 - 5 mg/ml, 2.3 – 10 mg/ml, 2.4 – 15mg/ml, 2.5 – 20mg/ml. 3. Ethyl acetate fraction - (3.1 - 2mg/ml, 3.2 - 5 mg/ml, 3.3 – 10 mg/ml, 3.4 – 15mg/ml, 3.5 – 20mg/ml. 4. Chloroform fraction - (4.1 - 2mg/ml, 4.2 - 5 mg/ml, 4.3 – 10 mg/ml, 4.4 – 15mg/ml, 4.5 – 20mg/ml. 5. DMSO fraction - (5.1 - 2mg/ml, 5.2 - 5 mg/ml, 5.3 – 10 mg/ml, 5.4 – 15mg/ml, 5.5 – 20mg/ml

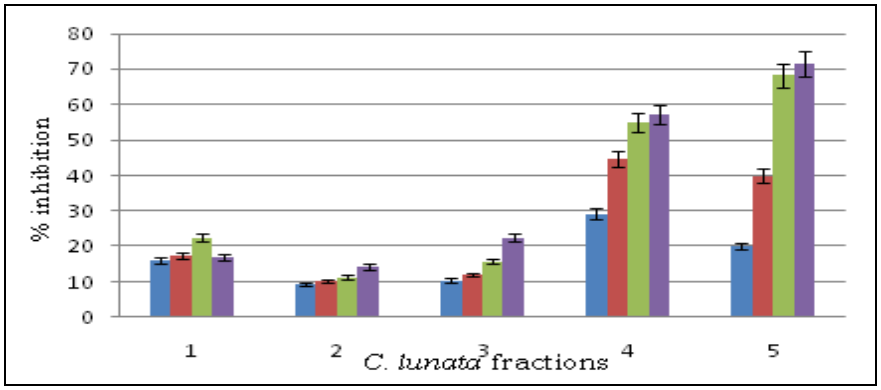

FIG. 8: ALPHA AMYLASE INHIBITION ACTIVITY. 1. DMSO fraction- (1.1 - 5 mg/ml, 1.2 – 10 mg/ml, 1.3 - 15mg/ml, 1.4 – 20mg/ml). 2. Methanol fraction - (2.1 - 5 mg/ml, 2.2 – 10 mg/ml, 2.3 – 15mg/ml, 2.4 – 20mg/ml). 3. Chloroform fraction - (3.1 - 5 mg/ml, 3.2 - 10 mg/ml, 3.3 - 15mg/ml, 3.4 – 20mg/ml). 4. Ethylacetate fraction - (4.1 - 5 mg/ml, 4.2 – 10 mg/ml, 4.3 – 15mg/ml, 4.4 - 20mg/ml). 5. Standard - Acarbose - (5.1 - 5 mg/ml, 5.2 - 10 mg/ml, 5.3 – 15mg/ml, 5.4 – 20mg/ml)

Determination of Glucose Uptake Capacity by Yeast Cells: The Ethyl acetate extract of *C. lunata* promoted the uptake of glucose across the plasma membrane of yeast cells. The glucose uptake at an initial concentration of 5 mM glucose by the extract was comparable to known drug metronidazole **Fig. 9**. However, the effect of metronidazole on glucose uptake by the yeast cells at 10 and 25 mM glucose concentration was a bit higher than that of extract. It was observed that by increasing the concentration of extract, yeast cells' capability to uptake more glucose from the environment also increases. The results concluded that the lower the concentration of glucose in the solution, the higher the uptake by yeast cells.

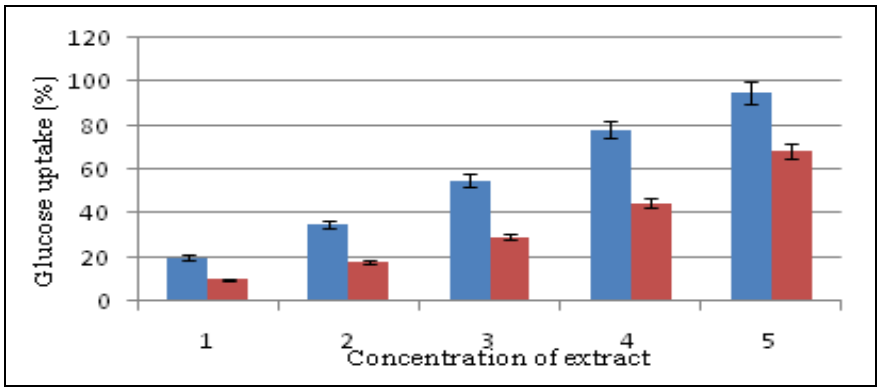

FIG. 9: GLUCOSE UPTAKE BY YEAST CELLS AT 5mM CONCENTRATION

Standard - Metronidazole - (1.1 - 2mg/ml, 2.1 - 5 mg/ml, 3.1 – 10 mg/ml, 4.1 – 15mg/ml, 5.1 – 20mg/ml)

Ethyl acetate fraction - (1.2 - 1mg/ml, 2.2 - 2 mg/ml, 3.2 – 3 mg/ml, 4.2 – 4mg/ml, 5.2 – 5mg/ml)

Anti-inflammatory Activity:

Inhibition of 15-Lipoxygenase (15-LOX) Enzyme: The anti-inflammatory activity was evaluated as % inhibition of LOX enzyme monitored as the formation of hydroperoxy linoleic acid at 234 nm. *C. lunata* extract showed dose-dependent inhibition. Ethyl acetate fraction exhibited maximum inhibition at 71%, while the standard NDGA had 93% inhibition at maximum concentration.

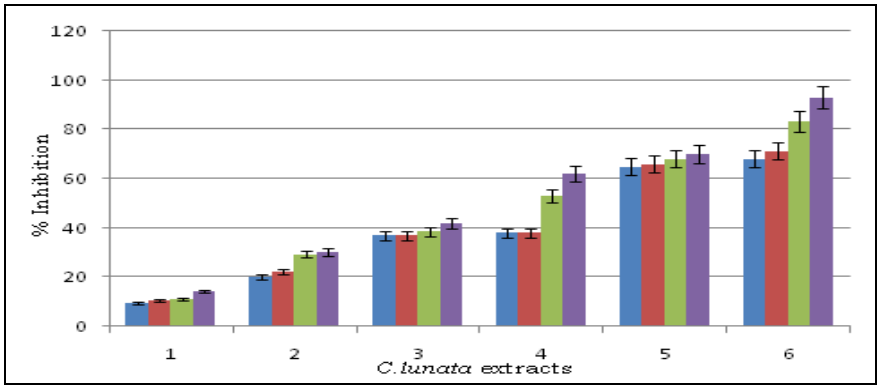

FIG. 10: 15-LOX INHIBITION ACTIVITY. 1. DMSO fraction (1.1 - 2mg/ml, 1.2 - 5 mg/ml, 1.3 – 10 mg/ml, 1.4 – 15mg/ml, 1.5 – 20mg/ml). 2. Methanol fraction - (2.1 - 2mg/ml, 2.2 - 5 mg/ml, 2.3 – 10 mg/ml, 2.4 – 15mg/ml, 2.5 – 20mg/ml). 3. Chloroform fraction - (3.1 - 2mg/ml, 3.2 - 5 mg/ml, 3.3 – 10 mg/ml, 3.4 – 15mg/ml, 3.5 – 20mg/ml). 4. Ethyl acetate fraction - (4.1 - 2mg/ml, 4.2 - 5 mg/ml, 4.3 – 10 mg/ml, 4.4 – 15mg/ml, 4.5 – 20mg/ml). 5.Quercetin - (5.1 - 2mg/ml, 5.2 - 5 mg/ml, 5.3 – 10 mg/ml, 5.4 – 15mg/ml, 5.5 – 20mg/ml)

Statistical Analysis: All the experiments were carried out in triplicate, and the data was analyzed by *t*-test using Microsoft Excel 2010. The error bars in the graphs represent ± S E of triplicate data with P-value at 0.05.

DISCUSSION: The work envisaged was to establish the bioactive potential of endophytic fungus *Curvularia lunata*. As an initial investigation the fungal extracts were tested for the presence of phytochemical constituents. On observing the presence of major secondary metabolites, it was further worked upon to test the potential of secondary metabolites present in the fungal extracts. The first extraction (to obtain secondary metabolites) was done using Ethyl acetate solvent, as it is reported to isolate a range of metabolites. The ethyl acetate extract was further fractioned using a column employing solvents from the polarity range - Chloroform, Dimethyl sulphoxide, and methanol. A total of four fractions (Chloroform, DMSO, Methanol, and Ethyl acetate) were employed, and from the results above, it can be fairly established that Ethyl acetate fraction is showing comparatively greater activity when tested using standard compounds/drugs.

This work also reimposes the fact that ethyl acetate is a good solvent to isolate a range of metabolites from fungi. Through this study, *Curvularia lunata* isolated from leaves of *Ficus religiosa* was found to exhibit anti-oxidant, anti-inflammatory, and anti-diabetic properties when tested in-vitro.

CONCLUSION: In conclusion, the study provides an overview of the role of endophytes in providing newer compounds or drug leads for treating certain medical disorders. The ability of the extracts to donate protons to stabilize the free radicals shows us the lead that metabolites present in extract have anti-oxidant property.

According to the results obtained, the ethyl acetate extracts from *C. lunata* demonstrated moderate inhibitory activity against pancreatic α-amylase and α-glucosidase. Inhibition of carbohydrate hydrolysing enzymes will delay the process of glucose breakdown in the food and thereby prevents rise in postprandial glucose levels or hyperglycaemia in the body. Similarly, a moderate inhibitory activity against the 15-LOX enzyme using the extracts suggests the possible anti-inflammatory potential of *C. lunata*.

In conclusion, it can be understood that secondary metabolites present in the extracts, such as flavonoids, phenols, saponins, and alkaloids, may be responsible for the observed activity. However, it is pertinent to mention that results obtained are performed as *in-vitro* experiments and need to be

confirmed by *in-vivo* tests (either in appropriate animal models or randomized clinical studies). There is a further need to evaluate the toxicological properties of these extracts. Elucidation of the mode of inhibition and structural characterization of the bioactive fractions and QSAR studies are also necessitated.

ACKNOWLEDGEMENT: Authors gratefully acknowledge the benediction of the Founder Chancellor of Institute, Bhagawan Sri Sathya Sai Baba. The authors are grateful to UGC SAP DRS level II funding for infrastructural support and to UGC for providing BSR Fellowship in Sciences.

CONFLICTS OF INTEREST: The Authors have no conflict of interest to declare.

REFERENCES:

1. DeBary A: Morphologieund Physiologieder Pilze, Flechten, und Myxomyceten: Hofmeister's Handbook of physiological Botany. Leipzig: W. Engelmann 1866.
2. Stone JK, Bacon CW and White JF: An Overview of Endophytic Microbes: Endophytism Defined. Microbial Endophytes 2000; 3-29.
3. Strobel GA: Endophytes as source of bioactive products. Microbes Infect 2003; 5(6): 535-44.
4. Zhang YJ, Zhang S, Liu XZ, Wen HA and Wang M: A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Letters in Applied Microbiology 2010; 51: 114-18.
5. Song R, Wang J, Sun L, Zhang Y, Ren Z, Zhao B and Lu H: The study of metabolites from fermentation culture of *Alternaria oxytropis*. BMC Microbiol 2019; 19(35): 1-8.
6. Sharma P, Khandelwal S, Singh T and Vijayvergia R: Phytochemical analysis and antifungal potential of *Duranta erecta* against some Phytopatogenic Fungi. Int J Pharm Sci Res 2012; 3(7): 2686-89.
7. Katta J, Rampilla V and Mohamad KS: Evaluation of phytochemical and pharmacological aspects of epiphytic orchid *Luisia zeylanica* Lindl. Int J Pharm Sci & Res 2020; 11(3): 1333-49.
8. Vattem DA, Lin YT, Labbe RG, Shetty K: Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food grade fungus Lentinus Edodes and effect on antimicrobial activity against select food borne pathogens. Innov Food Sci Emerg 2004; 5(1): 81-91.
9. Dulf FV, Vodnar DC, Dulf EH and Pintea A: Phenolic compounds, flavonoids, lipids and antioxidant potential of apricot (*Prunus armeniaca* L.) pomace fermented by two filamentous fungal strains in solid state system. Chem Cent J 2017; 11: 92.
10. Yadav M, Yadav A and Yadav JP: *In-vitro* antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. Asian Pacific Journal of Tropical Medicine 2014; 7(Suppl): S256-S261
11. Khalil DMA, El-Zayat SA Lentinusedodes El-Sayed MA: Phytochemical screening and antioxidant potential of endophytic fungi isolated from *Hibiscus sabdariffa*. J Appl Biotechnol Rep 2020; 7(2): 116-12
12. Kidane Y, Bokrezion T, Mebrahtu J, Mehari M, Gebreab YB, Fessehaye N and Achila OO: *In-vitro* Inhibition of α-amylase and α-glucosidase by extracts from *Psiadia punctulata* and *Meriandra bengalensis*. Evidence Based Complementary and Alternative Medicine 2018; 21643-45.
13. Pavithra N, Sathish L, Babu N, Venkatarathanamma V, Pushpalatha H, Reddy GB and Ananda K: Evaluation of α-amylase, α-glucosidase and aldose reductase inhibitors in ethyl acetate extracts of endophytic fungi isolated from anti-diabetic medicinal plants. Int J Pharm Sci Res 2014; 5(12): 5334-41.
14. Rehman G, Hamayun M, Iqbal A, Islam S, Arshad S, Zaman K, Ahmad A, Shehzad A, Hussain A and Lee IJ: *In-vitro* antidiabetic effects and antioxidant potential of *Cassia nemophila* Pods. BioMedResearc International 2018; 1824790: 1-6.
15. Karmakar R, Kumar S and Prakash HS: Bioactive potential of endophytic Myrothecium sp. isolate M1-CA-102, associated with *Calophyllum apetalum*. Pharm Biol 2014; 52(6): 665-76.
16. Dugan FM: The Identification of Fungi: An Illustrated Introduction with Keys, Glossary, and Guide to Literature. APS Publication 2017.
17. Lini IF, Afroz F, Begum N, Rony SR, Sharmin S, Moni F and Sohrab MH: Identification and bioactive potential of endophytic fungi from marine weeds available in the coastal area of Bangladesh. Int J Pharm Sci & Res 2020; 11(3): 1249-57.
18. Jeewon R, Luckhun AB, Bhoyroo V, Sadeer NB, Mahomoodally MF, Rampadarath S, Puchooa D, Sarma VV, Durairajan SS and Hyde KD: Pharmaceutical potential of marine fungal endophytes. Endophytes and Secondary Metabolites 2019: 283-05.

How to cite this article:
Jayant KK and Vijayakumar BS: *In-vitro* bioactive potential of endophytic fungi *Curvularia lunata* isolated from medicinal plant *Ficus religiosa*. Int J Pharm Sci & Res 2021; 12(8): 4322-30. doi: 10.13040/IJPSR.0975-8232.12(8).4322-30.