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Original paper

In-vitro anti-oxidant and anti-diabetic potential of endophytic fungi associated with *Ficus religiosa*

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Abstract
Endophytic fungi are capable of producing plethora of important secondary metabolites, previously known from medicinal plants, thereby raising the potential of exploring endophytes as alternate sources for these metabolites. In the present investigation, ten endophytic fungi were isolated from *Ficus religiosa* L. (Moraceae) and the extracts obtained from fungi were evaluated for their potential as anti-oxidant and anti-diabetic agents. Phytochemical screening of extracts revealed presence of phenols, flavonoids, alkaloids, steroids, terpenes and terpenoids. Anti-oxidant and anti- diabetic activities of the extracts were tested by DPPH free radical scavenging assay and alpha amylase inhibitory assay, respectively. In general, all the extracts showed moderate inhibition of DPPH thereby providing an insight that extracts have a good proton-donating ability and could be potential free radical inhibitors. Similarly, moderate inhibition of alpha amylase was shown by all the extracts suggesting their possible role in reducing the breakdown and flow of glucose from complex dietary carbohydrates into the bloodstream. Among the ten fungi tested, *Curvularia lunata* displayed the highest anti-oxidant and anti-diabetic activities.

Keywords
Endophytes, medicinal plant, secondary metabolites, phytochemicals, anti-oxidants, alpha-amylase inhibitors

Introduction
Endophytic fungi inhabit the host plant by living in their tissues and exhibit a mutual relationship with the plant without causing any disease symptom (Bacon and White, 2000). According to the plant-endophyte coevolution hypothesis suggested by Ji et al. (2009), it is believed that endophytes assist the plants in chemical defense by producing bioactive secondary metabolites. Since endophytes reside within plants and are in continuous interaction with their hosts, it is fairly relatable that plants would have a substantial influence in the metabolic processes of the endophytes. Occasionally, the compounds produced by endophytic fungi are similar as those produced by the plants (Kusari et al., 2012). Several metabolites produced by endophytic microorganisms have varied pharmaceutical applications and are used in production of anti-microbial, anti-fungal, anti-oxidant, anti-diabetic, anti-inflammatory and many such other potential lead drug compounds (Manganyi et al., 2020).

Ficus religiosa L. (Moraceae) is a large, perennial tree commonly planted in the temple areas and is considered sacred, especially in Southeast Asia (Government of India, 2016). Various parts of *F. religiosa* were assessed for their therapeutic qualities and found to have significant medicinal properties (Singh et al., 2011).

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It is a universal understanding that oxygen is major component of human existence. However in certain cases, this oxygen may be killer of cells when it generates reactive species that causes necrosis and untimely cell death. Reactive oxygen species (ROS) along with Reactive nitrogen species (RNS) causes oxidation by disrupting the physiological processes in the cell (Weseler and Bast, 2010). Free radicals such as ROS and others are generated as a result of biological and metabolic reactions in the body and also exogenous factors. Many human degenerative disorders such as diabetes mellitus, Alzheimer's, Parkinson's disease, atherosclerosis and inflammatory diseases are as a result of oxidative stress and membrane damage (Uttara et al., 2009). An 'antioxidant' can be labeled for any molecule/substance which inhibits or delays the oxidation of a substrate. There are several molecules either synthesised endogenously or exogenously consumed which play a role in antioxidant defense (Somogyi et al., 2007). There are many antioxidant compounds produced by endophytic fungi, for example, pestacin and isopestacin was obtained from *Pestalotiopsis microspora* (Speg.) G.C. Zhao & Nan Li, an endophytic fungus isolated from medicinal plant *Terminalia morobensis* Coode (Strobel and Daisy, 2003). Endophytes from *Passiflora incarnata* L. have been reported to produce phenolic compounds, methoxymethylphenol, orcinol and sorbicillin which have notable antioxidant activities in vitro and can be regarded as promising sources of new drugs (da Silva et al., 2020).

Diabetes mellitus and in specific type-II diabetes is emerging as a grave medical consideration in global scenario. It is estimated that about 422 million people worldwide have diabetes and about 1.6 million deaths in the world are directly attributed to diabetes each year (Roglic, 2016). This alarming situation demands research on novel therapeutic agents to treat diabetes. One of the major challenge, among many others, in diabetes treatment is normalising the blood glucose levels in patients. A potential preventive or control measure to achieve this is by controlling monosaccharide absorption and regulating the action and network of carbohydrate degrading/hydrolysing enzymes. Two principle digestive enzymes α-amylase and α-glucosidase are responsible for the degradation of complex dietary carbohydrates into sugar in the digestive tract. Regulation of these enzymes provides scope for lowering post-prandial rise in blood glucose levels and thereby bringing the insulin action/regulation under control (Alqahtani et al., 2019). Previous work on anti-diabetic potential of endophytic fungi isolated from *F. religiosa* showed that *Aspergillus* species among five other fungal isolates exhibited highest percentage of inhibition for α-amylase inhibition assay, α-glucosidase inhibition assay and showed good results in glucose diffusion assay. These results gave an indication about the hypoglycemic activity of the endophytic fungal crude extract (Tiwari et al., 2017).

With this background, this study attempts to report more endophytic fungi associated with *F. religiosa* and to evaluate their phytochemical and pharmacological potential in the context of oxidative stress and diabetes.

Materials and Methods

Collection of plant samples and culturing endophytic fungi

Fresh and healthy leaf samples of *F. religiosa* (identified by Botanical Survey of India, Regional Centre, Hyderabad) were collected from Puttaparthi, Andhra Pradesh, India during the year 2018. Composite sampling technique was used: multiple temporally or spatially discrete leaf samples were collected and combined, thoroughly homogenised, and treated as a single sample. The plant specimens were processed by thoroughly rinsing with tap water (Hallmann et al., 2006). Samples were cut into 3-4 cm pieces prior to 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 (PDA, Hi Media Laboratories) plates supplemented with streptomycin (250 mg L^{-1}). The efficacy of surface sterilization was confirmed by inoculating the surface sterilized water collected from last wash of the sample in a nutrient medium. The absence of growth of any fungi

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on the media confirms the efficient surface sterilization of the segments. The inoculated petri plates were sealed and incubated at 25 ^{O}C in an incubation chamber. After 3 days of inoculation, the plates were observed daily for growth of the fungi from cultured segments up to two weeks. The fungus that emerged from tip of the segment was picked up, sub-culturedand the pure culture was maintained on PDA for the extraction of fungal metabolites.

Phenotypic and genotypic identification of the endophytic fungi

The isolated endophytic fungi were initially identified by microscopic examination (Biological binocular microscope, Dewinter Optical Inc, New Delhi, India) of colony morphology and reproductive characteristics using slide cultures. Morphological identification of the endophytic fungi was carried out at Indian Type Culture Collection Centre, Division of Plant Pathology, IARI, New Delhi.

For genotypic identification of the fungus showing high bioactivity, total genomic DNA of the endophytic fungus was isolated directly from actively growing mycelium growing in potato dextrose broth (PDB). Isolation of DNA and the subsequent steps of Polymerase chain reaction (PCR) and DNA sequencing were done by the Pentavalent Biotechnology Pvt. Ltd., Bengaluru, India. Fungal DNA was extracted using the protocol of Zhang et al. (2010) and the extracted DNA was amplified using ITS1 – ITS4 primers (White et al., 1990). Amplified DNAs were sequenced and the obtained sequences were compared with already existing DNA sequences in NCBI Gen Bank (http://www.ncbi.nlm.nih.gov.blast) to identify the respective fungi.

Extraction of fungal metabolites

The mycelia from growing colony edge were inoculated into PDB, for production of fungal metabolites by fermentation method. Mycelia were inoculated into 1000 mL

Erlenmeyer flask containing 500 mL of PDB medium and kept on a rotary shaker (Orbital shaker, Scigenics, Chennai, India) for 14 days at 25 ^{O}C. Each culture was filtered using Whatman filter paper. Solvent extraction procedure using ethyl acetate as organic solvent was employed to obtain fungal crude extracts. To the culture filtrate equal volume of ethyl acetate (AR grade, 99% pure, Merck) was added, mixed well for 10 min and kept aside for 5 min till the two clear immiscible layers formed. The upper layer of solvent containing the extracted compounds was separated using separating funnel (Bhardwaj et al., 2015), and evaporated using rotary vacuum evaporator (Heidolph, model: 571-01300-06-0 Hei-VAP Core HL G3) to yield the crude dry extracts (yield obtained ranged from 1.2 mg to 2 mg for ten fungal cultures). The crude extracts were then dissolved in ethyl acetate at various concentrations (0.1-0.5 mg mL^{-1}) and kept at 4 °C.

Qualitative phytochemical analysis

The endophytic fungal extracts were tested for the presence of the secondary metabolites such as alkaloids, phenols, flavonoids, steroids and terpenoids by various qualitative techniques (Harborne, 1998).

Alkaloids

The endophytic fungal crude extract (0.5 mg mL^{-1}) was evaporated and the residues were dissolved in 2 N HCl. The mixture was filtered and the filtrate was used for the following test:

• Wagner's test: reddish brown precipitate formation indicated presence of alkaloids on addition of Wagner's reagent (Tiwari and Gupta, 2020).
• Hager's test: presence of alkaloid was confirmed with yellow colour precipitate on addition of Hager's reagent (Tiwari and Gupta, 2020).

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Flavonoids

• Alkaline reagent test:fungal extract was treated with 10% ammonium hydroxide solution. Presence of flavonoids was detected by a yellow fluorescence (Tiwari and Gupta, 2020).

• 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 boiling solution for few minutes. In the presence of flavonoids, pink or dirty brown colour was observed (Tiwari and Gupta, 2020).

Phenols

• Ferric chloride test: extract was treated with 5% ferric chloride solution. Dark green colour indicated the presence of phenol (Devi et al., 2012).

• Lead acetate test: 10% lead acetate solution was treated with fungal crude extract and a bulky white colour indicated phenolic presence (Vimalkumar et al., 2014).

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 indicated the presence of the steroidal ring (Tiwari and Gupta, 2020).

Terpenoids

• The Libermann–Burchard test: a deep red layer at the junction of two layers indicated the presence of terpenoids, when 1 mL of the fungal crude extract was treated with few drops of acetic acid and 1 mL concentrated sulphuric acid (Tiwari and Gupta, 2020).

Total phenolic content

Total phenolic content of ethyl acetate extracts of endophytic fungi was estimated by Folin-Ciocalteau reagent based assay using gallic acid as standard (Halliwel and Guttridge, 1989). Total phenolic value was obtained from the regression equation: $y = 0.0009X + 0.0686$ with $R^2 = 0.999$ and expressed as mg of gallic acid equivalents (GAE)/g of extract (dw).

Anti - oxidant activity of fungal extracts

DPPH free radical scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable, nitrogen-centred free radical which produces violet colour in methanol solution. Change in absorbance of DPPH was monitored for anti-oxidant activity through free radical scavenging. DPPH free radical scavenging assay is considered to be one of the basic, accurate, and widely used methods to find out the antioxidant potential of various natural products (Brand-Williams et al., 1995). Endophytic fungal extracts at a concentration range of 0.1 – 0.5 mg mL^{-1} were used. DPPH solution (0.5 mM L^{-1}) was prepared in 95% methanol and a total of 2 mL of DPPH solution was added to the test sample (200 µL) and incubated for 30 min at room temperature in darkness. After incubation, the absorbance was measured at 517 nm (Manzocco et al., 1998). Ascorbic acid was taken as standard. Free radical scavenging activity was expressed as percentage inhibition which was calculated as:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \text{ X } 100$$

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Inhibition of carbohydrate digestive enzyme (Anti-diabetic activity)

Alpha amylase Inhibitory (AAI) Assay

Reaction mixture was prepared by adding 40 µL of fungal extract (0.1 – 0.5 mg mL^{-1}), 200 µL of phosphate buffer (pH 6.9), and 40 µL (24 U mL^{-1}) 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. Reaction was terminated by adding 0.5 mL DNS (3,5-dinitrosalicylic acid) 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 buffer. Control representing enzyme activity was prepared in a similar manner without extract. Acarbose was used as standard and compared with the test samples. The experiments were repeated thrice using the same protocol (Nair et al., 2013).

The AAI activity was calculated using the formula:

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

Results

Morphological and genetic identification of endophytic fungi

Ten endophytic fungi were isolated from F. *religiosa* and identified by morphological features and ITS gene sequencing. The cultures obtained were *Aspergillus aculeatus* Iizuka*, Penicillium* sp., *Aspergillus sydowii* (Bainier & Sartory) Thom & Church*, Curvularia lunata* (Wakker) Boedijn (NCBI accession number: MT825246)*, Cephaliophora irregularis* Thaxt.*, Diaporthe* sp., *Aspergillus quadrilineatus* Thom & Raper*, Aspergillus flavus* Link*, Aspergillus versicolor* (Vuill.) Tirab. and *Aspergillus* sp. (Taxonomic citation source: http://www.indexfungorum.org/names/names.asp).

Phytochemical analysis and determination of total phenolic content

The ethyl acetate extracts of endophytic fungi revealed presence of alkaloids, phenols, flavonoids, saponins, terpenes, terpenoids and steroids as depicted in Table 1. Total phenolic content was estimated and the highest concentration of phenolics was observed in the extract of *C. lunata*, equal to of 21.7 ± 0.19 mg GAE/g of extract (dw).

Anti-oxidant activity

DPPH free radical scavenging assay

The reaction was visible as a color change from purple to yellow. Ethyl acetate extract of *C. lunata* at a concentration of 0.5 mg mL^{-1} showed the best anti-oxidant capacity (inhibitory % of 90.7 ± 1.0%). Ascorbic acid asstandard exhibited 95.95 ± 1.45% inhibitory activity (Fig. 1). The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The result showed that the extract has a good proton-donating ability and could be a potential free radical inhibitor. The IC_{50} values of the standard and *C. lunata* extract (showing high inhibitory %) were calculated (https://www.aatbio.com/tools/ic50-calculator) and are as depicted in Tables 2 and 3, respectively.

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Table 1 - Phytochemicals present in the fungal extracts and total phenolic content (mg GAE/g of extract [dw]).

Fungal endophyte	Phytochemicals detected							Total Phenolic Content
	Alkaloids	Phenols	Flavonoids	Steroids	Terpenes	Terpenoids	Saponins	[mg GAE/g of extract (dw)]
Aspergillus aculeatus	+	+		+				13.78 ± 0.13
Penicillium sp.	+	+	+					13.14 ± 0.06
Aspergillus sydowii	+	+			+			12.4 ± 0.05
Curvularia lunata	+	+	+	+	+	+	+	21.7 ± 0.19
Cephaliophora irregularis	+	+					+	0.80 ± 0.10
Diaporthe sp.	+	+	+				+	0.78 ± 0.09
Aspergillus quadrilineatus	+	+				+		1.12 ± 0.19
Aspergillus flavus	+	+			+			1.39 ± 0.22
Aspergillus versicolor	+	+					+	1.62 ± 0.08
Aspergillus sp.	+	+		+				2.42 ± 0.04

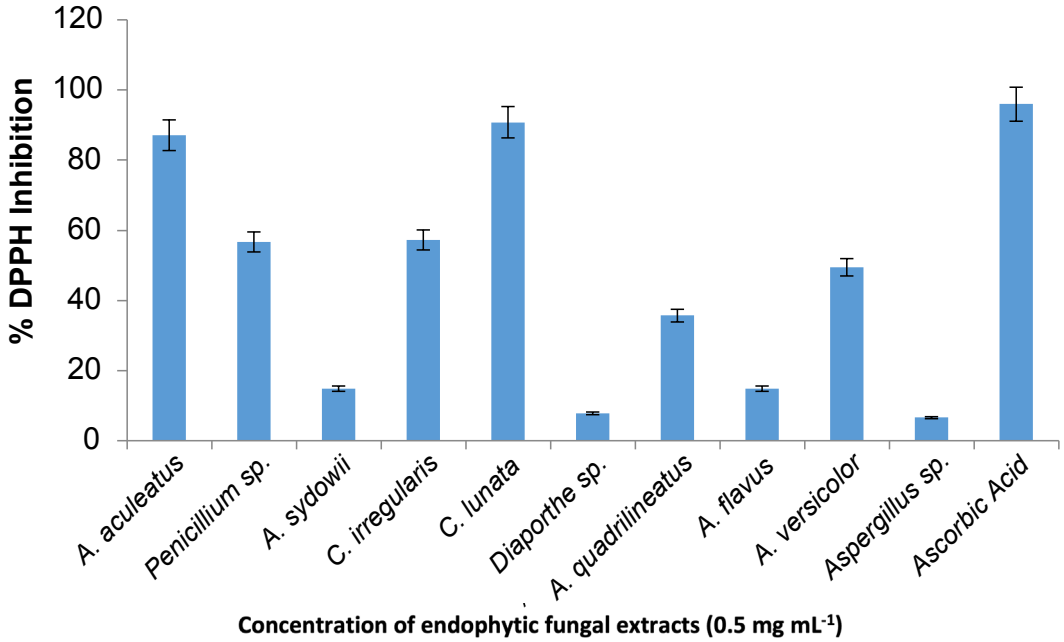

Fig. 1 - DPPH Inhibition by fungal extracts. Each bar represents particular fungus and the last bar is % inhibition of ascorbic acid (standard).

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Table 2 - DPPH % Inhibition and IC_{50} value of Ascorbic Acid (Standard).

AscorbicAcid (Standard)	Concentration (mg mL^{-1})	% Inhibition	IC_{50} value (mg mL^{-1})
	0.1	35.67 ± 0.94	0.217± **0.**75
	0.2	58.32 ± 0.77	
	0.3	87.16 ± 0.16	
	0.4	91.77 ± 0.43	
	0.5	95.95 ± 1.45	

Table 3 - DPPH% Inhibition and IC_{50} value of *C. lunata* extract (showing high % inhibition).

Ethyl acetate extract of *C. lunata*	Concentration (mg mL^{-1})	% Inhibition	IC_{50} value (mg mL^{-1})
	0.1	7.88 ± 0.03	0.42 ± **0.**54
	0.2	14.90 ± 0.23	
	0.3	22.29 ± 0.65	
	0.4	57.27 ± 0.79	
	0.5	90.77 ± 1.0	

Anti-diabetic Activity of fungi

α-*Amylase inhibition (AAI) assay*

Antidiabetic activity of fungal extracts was determined by inhibition of α-amylase. All the extracts showed enzyme inhibitory activity and the *C. lunata* extract was the most effective in this assay also (Fig. 2). Ethyl acetate extract of *C. lunata* at a concentration of 0.5 mg mL^{-1} exhibited an inhibition of 80.4%. Standard α-amylase inhibitor - Acarbose showed an inhibitory activity of 98.8%. The IC_{50} values of the standard and *C. lunata* extract (showing high inhibitory %) were calculated (https://www.aatbio.com/tools/ic50-calculator) and are as depicted in Tables 4 and 5 respectively.

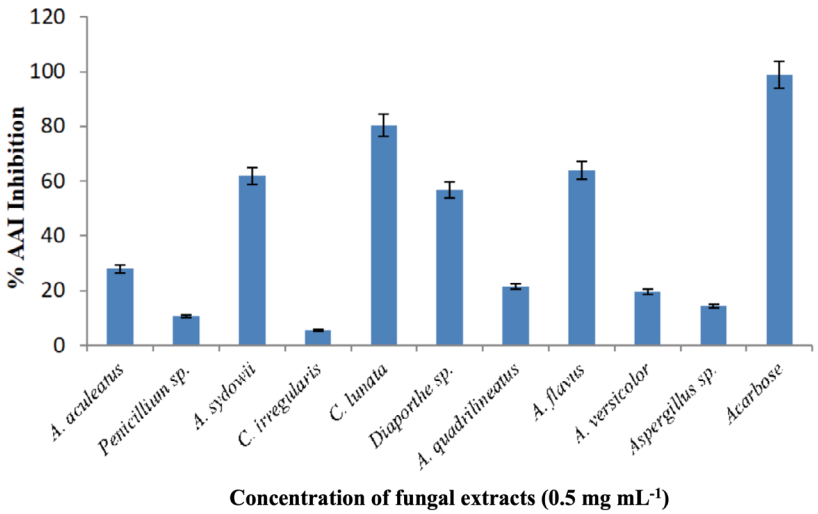

Fig. 2 - Alpha amylase inhibition by fungal extracts. Each bar represents particular fungus and the last bar is % inhibition of acarbose (standard).

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Table 4 - Alpha amylase% Inhibition and IC_{50} value of Acarbose (Standard).

Acarbose (Standard)	Concentration (mg mL^{-1})	% Inhibition	IC_{50} value (mg mL^{-1})
	0.1	10.8 ± 0.08	0.3187 ± 0.24
	0.2	21.6 ± 0.05	
	0.3	56.8 ± 0.20	
	0.4	80.4 ± 0.36	
	0.5	98.8 ± 0.55	

Table 5 - Alpha amylase % Inhibition and IC_{50} value of *C. lunata* extract (showing high % inhibition).

Ethyl acetate extract of *C. lunata*	Concentration (mg mL^{-1})	% Inhibition	IC_{50} value (mg mL^{-1})
	0.1	5.6 ± 0.44	0.3563± 0.46
	0.2	14.4 ± 0.24	
	0.3	28 ± 0.4	
	0.4	64 ± 0.67	
	0.5	80.4 ± 0.56	

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 mean of triplicate data ± standard error with $P \leq 0.05$. The data represents average of three independent experiments carried out with three replicates from an experiment.

Discussion and Conclusions

Ten endophytic fungal strains were isolated from leaves of *F. religiosa.* Strains of the genus *Aspergillus* were found predominantly. Other endophytic fungi from the genus *Penicillium, Curvularia, Cephaliophora* were found in *F. religiosa*.

Fungal extracts were obtained using ethyl acetate as solvent, asethyl acetate extraction is reported to be most efficient method of isolating secondary metabolites from fungal species (Yadav et al., 2014). The qualitative phytochemical analysis confirmed the presence of phenols, flavonoids, alkaloids, terpenoids and steroids, varyingamong the different fungal extracts. The extracts were further evaluated for antioxidant and anti-diabetic activities.

The α-amylase inhibition assay performed as part of this study demonstrated that all the fungal extracts showed activity and the ethyl acetate extract of *C. lunata* had significant inhibitory potential. The IC_{50} value (0.35± 0.46 mg mL^{-1}) of *C. lunata* extractis comparable to that of Acarbose (0.31 ± 0.24 mg mL^{-1}), a widely used and marketed anti-diabetic drug. Previously, Tiwari et al. (2017) had isolated twenty-one endophytic fungi from leaf explants of *F. religiosa* and these were evaluated for anti-diabetic or hypoglycemic activities. Among these nine pure endophytic cultures were isolated and five tested for alpha amylase inhibition and alpha glucosidase inhibition. Petroleum ether extract of *Aspergillus* sp. showed highest inhibitory action against alpha amylase and alpha glucosidase enzymes. Lower concentration (100 µg) of crude extract showed maximum inhibitory activity with 91 ± 0.06% for alpha amylase and 43 ± 0.01% for alpha glucosidase enzymes. The endophytic fungus *Dendryphion nanum* (Nees) S. Hughes isolated from leaves of *F. religiosa* yielded a naphtoquinone

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compound which induced glucose uptake in rat skeletal muscles in the presence of insulin with an EC_{50} of 0.80 ± 0.090 µM. Rosiglitazone, a known glucose uptake activator (EC_{50} = 3.0 ± 0.040 µM) was used as a standard in the assay. The ability of compound to induce glucose uptake at such low concentration indicated that it could be a good scaffold as a starting point for drug development (Mishra et al., 2013).

It is predicted that diabetic complications occur as a result of the oxidative stress due to the formation of free radicals with the glucose oxidation (Mehta et al., 2006). Therefore, the use of antioxidants along with anti-diabetic drugs is frequently recommended to avoid such complications. The antioxidant activities measured by DPPH scavenging assay and the total phenolic content of the fungal extracts indicate that all extracts possess antioxidant properties with *C. lunata* exhibiting the best DPPH scavenging ability (IC_{50} of 0.42 ± 0.54), comparable to that of standard Ascorbic acid (IC_{50} of 0.21 ± 0.75 mg mL^{-1}), and the highest total phenolic content (21.7 ± 0.19 mg GAE/g extract). Our findings support some of the previous studies, such as that of Yadav et al. (2014) which have reported about the relationship between total phenolic content and antioxidant activity of ethyl acetate extracts of endophytic fungi.

Literature on *Curvularia* sp. isolated from various medicinal plants suggests that about 143 secondary metabolites were purified from their crude extracts. These metabolites belonged to several chemical classes such as alkaloids, anthraquinones, polyketides, quinones, terpenes and peptides. Several isolated compounds showed diverse biological activities including antimalarial, antifouling, antilarval, anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal, anti-cancer, leishmanicidal properties and phytotoxicity (Khiralla et al., 2019). The new endophytic fungus *Curvularia* sp. T12 was isolated from the medicinal plant *Rauwolfia macrophylla* Stapf. Its large scale fermentation and working up of the crude extract, led to the isolation of 2'-deoxyribolactone, hexylitaconic acid and ergosterol. These compounds displayed antimicrobial, antioxidant and acetylcholinesterase inhibition activities (Kaaniche et al., 2019).

In conclusion the study provides an overview of the role of endophytes in providing new compounds or leads for treating certain medical disorders. *Curvularia lunata* isolated from *F. religiosa* showed greater anti-oxidant and anti-diabetic potential and this can be attributed to various phytochemicals detected in the ethyl acetate extract of the fungus. However, it is pertinent to mention that results obtained are performed as *in vitro* experiments and need to be confirmed by *in vivo* tests. Future direction for this study would be elucidation of the mode of inhibition and structural characterization of the bio-active fractions and QSAR studies. There is a further need to evaluate the toxicological properties of these extracts/compounds obtained from extracts and test for ADME properties.

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References

Alqahtani AS, Hidayathulla S, Rehman MT, El Gamal AA, Al-Massarani S, Razmovski-Naumovski V, Alqahtani MS, El Dib RA, AlAjmi MF (2019) Alpha-amylase and alpha-glucosidase enzyme inhibition and antioxidant potential of 3-oxolupenal and katononic acid isolated from *Nuxia oppositifolia*. Biomolecules 10(1):61. https://doi.org/10.3390/biom10010061

Bacon CW, White J (2000) Microbial endophytes. CRC press, Boca Raton.

https://doi.org/10.6092/issn.2531-7342/12104

Bhardwaj A, Sharma D, Jadon N, Agrawal PK (2015) Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinusroxburghii*. Archives of Clinical Microbiology 6 (3):19.

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT Food Science Technology 28:25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

da Silva M, Cueva-Yesquén LG, Júnior SB, Garcia VL, Sartoratto A, de Angelis DF, de Angelis DA (2020) Endophytic fungi from *Passiflora incarnata*: an antioxidant compound source. Archives of microbiology 202(10):2779–2789. https://doi.org/10.1007/s00203-020-02001-y

Devi NN, Prabakaran JJ, Wahab F (2012). Phytochemical analysis and enzyme analysis of endophytic fungi from *Centella asiatica*. Asian Pacific Journal of Tropical Biomedicine 2:S1280–S1284. https://doi.org/10.1016/S2221-1691(12)60400-6

Government of India, Ministry of Ayush (2016) The ayurvedic pharmacopoeia of India. Pharmacopoeia Commission for Indian Medicine & Homoeopathy, pp. 17-20.

Halliwel B, Guttridge JM (1989) Free radicals in biology and medicine, 2nd ed. Clarendon Press, London.

Hallmann J, Berg G, Schulz B (2006) Isolation procedures for endophytic microorganisms. In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial Root Endophytes. Soil Biology, vol 9. Springer, Berlin, Heidelberg. https://doi.org/10.1007/3-540-33526-9_17

Harborne JB (1998) Phytochemical methods A guide to modern techniques of plant analysis. Chapman and Hall Limited, 3rd ed. London, pp 142-175.

Ji HF, Li XJ, Zhang HY (2009) Natural products and drug discovery. EMBO Reports 10:194-200. https://doi.org/10.1038/embor.2009.12

Kaaniche F, Hamed A, Abdel-Razek AS, Wibberg D, Abdissa N, El Euch IZ, et al. (2019) Bioactive secondary metabolites from new endophytic fungus *Curvularia*. sp isolated from *Rauwolfia macrophylla*. PLoS ONE 14(6), e0217627. https://doi.org/10.1371/journal.pone.0217627

Khiralla A, Spina R, Saliba S, Laurain-Mattar D (2019) Diversity of natural products of the genera *Curvularia* and *Bipolaris*. Fungal Biology Reviews 33:101-122. https://doi.org/10.1016/j.fbr.2018.09.002

Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chemistry & Biology 19(7):792–798. https://doi.org/10.1016/j.chembiol.2012.06.004

Manganyi MC, Ateba CN (2020) Untapped potentials of endophytic fungi: A review of novel bioactive compounds with biological applications. Microorganisms 8(12):1934. https://doi.org/10.3390/microorganisms8121934

Manzocco L, Anese M, Nicoli MC (1998) Antioxidant properties of tea extracts as affected by processing. LWT - Food Science and Technology 31(7-8):694-698. https://doi.org/10.1006/fstl.1998.0491

Mehta JL, Rasouli N, Sinha AK, Molavi B (2006) Oxidative stress in diabetes: a mechanistic overview of its effects on atherogenesis and myocardial dysfunction. International Journal of Biochemistry and Cell Biology 38(5-6):794–803. https://doi.org/10.1016/j.biocel.2005.12.008

Mishra PD, Verekar SA, Kulkarni-Almeida A, Roy S K, Jain S, Balakrishnan A, Vishwakarma R, Deshmuk SK (2013) Anti-inflammatory and anti-diabetic naphthoquinones from an endophytic fungus *Dendryphion nanum* (Nees) S. Hughes. Indian Journal of Chemistry Section B 52:565-567.

Nair SS, Kavrekar V, Mishra A (2013) In-vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. European Journal of Experimental Biology 3(1):128-132.

https://doi.org/10.6092/issn.2531-7342/12104

Roglic G (2016) WHO Global report on diabetes: a summary. International Journal of Noncommunicable Diseases 1:3-8.

Singh D, Singh B, Goel R (2011) Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: a review. Journal of ethnopharmacology 134:565-83. https://doi.org/10.1016/j.jep.2011.01.046

Somogyi A, Rosta K, Pusztai P, Tulassay Z, Nagy G (2007) Antioxidant measurements. Physiological measurement 28(4):R41–55. https://doi.org/10.1088/0967-3334/28/4/R01

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews 67(4):491–502. https://doi.org/10.1128/MMBR.67.4.491-502.2003

Tiwari P, Gupta R (2020) Preliminary phytochemical screening of bark (powder) extracts of *Ficus religiosa* (peepal) plant. International Journal of Research and Development in Pharmacy & Life Sciences 9(1):1-6. https://doi.org/10.47128/IJRDPL.2278-0238.2020.9(1).1-9

Tiwari P, Nathiya R, Mahalingam G (2017) Antidiabetic activity of endophytic fungi isolated from *Ficus Religiosa*. Asian Journal of Pharmaceutical and Clinical Research 10(4):59-61. https://doi.org/10.22159/ajpcr.2017.v10i4.14718

Uttara B, Singh AV, Zamboni P, Mahajan R (2009) Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Current Neuropharmacology 7(1):65–74. https://doi.org/10.2174/157015909787602823

Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG (2014) Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Olea dioica* Roxb., infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins and non-infected plants. Journal of Pharmacognosy and Phytochemistry 3(4):69-72.

Weseler AR, Bast A (2010) Oxidative stress and vascular function: implications for pharmacologic treatments. Current hypertension reports 12(3):154–161. https://doi.org/10.1007/s11906-010-0103-9

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA enes for phylogenetics. In: Innis, MA, Gelfand DH, Sninsky JJ, White TJ (eds.), PCR Protocols: A guide to methods and applications. Academic Press, New York, pp 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Yadav M, Yadav A, Yadav J P (2014) In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. Asian Pacific journal of tropical medicine 7(S1):S256–S261. https://doi.org/10.1016/S1995-7645(14)60242-X

Zhang YJ, Zhang S, Liu XZ, Wen HA,Wang M (2010) A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Letters in applied microbiology 51(1):114–118. https://doi.org/10.1111/j.1472-765X.2010.02867.x