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Antidiabetic activity of endophytic fungi isolated from *Aegle marmelos* and *Momordica charantia*

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Abstract: *Alloxan-induced oxidative stress has been demonstrated to harm beta-cells in the pancreas and result in hyperglycemia. In Ayurveda, Aegle marmelos leaf extract is used as a diabetes medication. The current study looked at ability of Aegle marmelos to prevent experimental diabetes and its antioxidant capacity. In those with alloxan diabetes, a methanolic extract of Aegle marmelos was proven to lower blood sugar levels. As advised by Indian Ayurveda, bilva has been widely used to treat diabetes and intestinal disorders. It is also frequently used to treat diabetes and other metabolic disorders. Karela, balsam pear, bitter melon, and bitter gourd. Indigenous communities in Asia, South America, India, and East Africa frequently employ Momordica charantia to treat diabetes and other metabolic disorders because of its ability to lower blood sugar levels. Through a variety of hypothesised processes, Momordica charantia has been shown to have positive effects in numerous pre-clinical trials. It was out that the endophytic fungi that were separated from the fruit and leaf samples were not the same. Each fungal species had a different morphological description. Based on their appearance, we discovered that the samples were surrounded by Aspergillus versicolor, Aspergillus niger, Penicillium verhagenii, and Fusarium oxysporum fungi. Each fungal species exhibited different levels of inhibitory properties towards the α-amylase, ranging from 53% to 30%, 36% to 19%.*

Key Words: *Antidiabetic Activity, Endophytic Fungi, α -amylase Inhibition, Fungal Metabolites, Natural Bioactive Compounds, Metabolite Extraction, Enzyme Inhibition.*

1. INTRODUCTION:

Diabetes mellitus, one of the most prevalent systemic diseases worldwide, is brought on by either insufficient insulin production or insulin resistance. According to the World Health Organisation, the number of people with diabetes mellitus, regardless of type, has increased dramatically over the past several decades worldwide, and by 2045, it is predicted to reach 629 million. There are still insufficient safe preventive medications available, despite the startlingly high frequency of diabetes mellitus. For many years, folkloric medicine has employed natural materials and compounds derived from bacteria, fungi, plants, and other living things to create a wide variety of natural formulations that heal a variety of ailments. Relatively little research has been done on endophytic fungus as the main source of bioactive metabolites such industrial enzymes, anticancer, antioxidant, antibacterial, and antidiabetic compounds. These fungi live inside plants without harming the host plant. The promise of endophytic fungal substances and extracts to prevent diabetes mellitus is summed up in this mini-review. The function of endophytic fungal extracts and their impact on diabetes mellitus have not been well studied [1].

SF-5060, isolated from Ethyl acetate extract of a culture broth of the marine-derived fungus *Cosmospora sp*, at inter-tidal sediment collected at Gejae Island, Korea, yielded the known compound *Aquastatin A*. The compound exhibited potent inhibitory activity against protein tyrosine phosphatase 1B (PTP1B) with an EC50 value of 0.19 mM. Tyrosine phosphorylation-dependent cellular processes are modulated by a broad family of enzymes known as protein tyrosine phosphatases (PTPs) [2]. Research has shown that PTP1B, an intracellular non-receptor type PTP, negatively controls signalling pathways mediated by insulin and leptin receptors. Therefore, blocking it could be a fantastic, cutting-edge treatment for type 2 diabetes and obesity. Kinetic analysis and testing over a small panel of different PTPs revealed that Aquastatin A inhibits PTP1B activity in a competitive and selective manner, respectively [3].

Oxidative stress is produced by the body's regular metabolic processes as well as also be brought on by a number of substances and environmental variables. It has been demonstrated that oxidative stress plays a major role in both the

aetiology of diabetes and its consequences in humans. It has been demonstrated that oxidative stress and a decline in antioxidant status coexist in diabetes. However, it is unknown exactly how oxidative stress contributes to the development of diabetes in humans. It has been demonstrated that oxidative stress causes changes in the structural functions of the collagen basement membrane, glycation of proteins, and inactivation of enzymes. The insulin receptor or the glucose transport protein (GLUT) may be significantly impacted by oxidative stress [4].

Native populations in Asia, South America, India, the Caribbean, and East Africa frequently employ *M. charantia*, also referred to as bitter melon, karela, balsam pear, or bitter gourd, as a remedy for diabetes-related ailments. This distinctive gourd has become recognised for its peculiar bitter flavour as well as which becomes more apparent with age.[5]. In Traditional Chinese Medicine, *M. charantia* has also been used for centuries to treat high blood sugar and the early stages of diabetes. Karela juice, which is made by crushing and straining the unripe fruits, and crease, a decoction of the plant's aerial parts, are the two most widely used ethnomedicinal preparations of the bitter gourd. These days, nutritional supplements in the form of pills and capsules containing powdered medications or extracts are sold over-the-counter and online [6].

Aegle marmelos, an indigenous plant of India, has been used by the Indian subcontinent's inhabitants for about 5000 years. To cure a wide range of illnesses, the Indian traditional medical system, Ayurveda, and diverse forms of folk medicine make extensive use of leaves, bark, roots, fruits, and seeds. Bael fruits are utilised in food, and its pulp is used to make sweet treats including juice, pudding and murabba. In several traditional treatments, bael fruits are also used as a laxative, to cure respiratory ailments, and to treat peptic ulcers, chronic diarrhoea, and dysentery [7]. Bioactive substances such coumarin, xanthotoxol, imperatorin, aegeline, and marmeline are found in bael's fruits, bark, leaves, seeds, and roots. These substances have antimicrobial, immunogenic, antifertility, anti-diabetic, anti-cancer, and insecticidal properties. A special fatty acid found in bael seeds, 12-hydroxyoctadec-cis-9-enoic acid or ricinoleic acid, can be converted into biodiesel [8].

By delving into the metabolic profiles of endophytic fungi associated with these plants, this research seeks to identify novel bioactive compounds with targeted actions on carbohydrate metabolism. Specifically, the study focuses on isolating metabolites capable of inhibiting key enzymes such as α-amylase, which play a pivotal role in starch breakdown and subsequent glucose release. By mitigating this enzymatic activity, these compounds hold potential for regulating postprandial glucose levels and providing a foundation for new antidiabetic therapies [9,10].

The objectives of this study include the isolation and characterization of endophytic fungi isolated from leaf and fruit sample of *Aegle marmelos and Momordica charantia*, solvent extraction of fungal metabolites, and evaluation of their antidiabetic activity through α-amylase inhibition assays. This research contributes to the growing field of bioprospecting, offering insights into the potential of fungal endophytes as sources of novel antidiabetic agents.

3. OBJECTIVES:

The objectives can be stated as:

- Isolation and characterization of endophytic fungi
- Solvent extraction of isolated fungi.
- Analysis of antidiabetic assay.

4. METHODOLOGY:

- **Materials:** The experimental work was conducted at the Department of Biosciences and Technology, Chhatrapati Sambhajinagar, during the academic year 2022–2023, providing a well-equipped and controlled environment for the research. The study utilized a range of essential glassware, including conical flasks, Petri plates, slides, test tubes, and tips, to facilitate the experimental procedures. Instruments such as an autoclave, laminar airflow cabinet, spectrophotometer, incubator, rotatory shaker, and microwave were employed to ensure precision and maintain sterile conditions throughout the experiments. A variety of chemicals, including ethanol, sodium hypochlorite, malt extract, glucose, peptone, agar, streptomycin sulphate, lactophenol cotton blue, dextrose, ethyl acetate, sodium phosphate buffer, and 3,5-dinitrosalicylic acid (DNSA), were used for the preparation of media, staining, and enzymatic assays.

- **Isolation of Endophytic fungi:** The plant leaves and stems were rinsed under running tap water, and leaf segments were cut evenly from the mid sections of healthy leaves, including the midrib, using a sterilised knife. Sterile dis. H_2O for 60 seconds, 70% ethanol for 60 seconds, 2.5% sodium hypochlorite for 4 minutes, 70% ethanol for 30 seconds, and a final rinse in sterile dis. H_2O three times were the solutions that were used to surface sterilise the cut

segments. To successfully sterilise the surface, 100 µL of the last rinse water was inoculated on Malt Extract Agar (MEA) medium (Oxoid, UK). Following sterilisation, the plant leaves were divided into five segments (5 mm), with 20 leaf segments per plant (five segments per plate) placed on the MEA plate surface. To prevent bacterial growth, 0.05 g of streptomycin sulphate was added to 100 mL of medium, and the plates were incubated at 28 °C ± 2 °C. Single isolates that had grown out of the tissues were re-inoculated on new MEA plates and kept in the MEA slant at 4 °C. The plates were examined every day for any fungal development.

- **Morphological characteristics of endophytic fungi:** Morphological characteristics were evaluated under a microscope. A drop of deionised water was added to the slide to prepare it, and the culture was removed from the petri plates using forceps. Using a toothpick, these colonies were put on the slides, combined with the liquid drop, and then covered with coverslips. A needle was used to secure cover slips on slides. Now, a microscope with a 100x zoom was being used to view the hyphae organisation, branching structure, and sporulation.

- **Production of anti-diabetic metabolites from endophytes:** For primary screening, 50 ml of malt extract broth (MEB) containing 3% malt extract, 0.5% peptone, and 2% dextrose was produced in Erlenmeyer flasks (250 mL). Using a sterile borer or tips, a tiny block of agar (6 mm in diameter) from PDA plates that had endophytic fungal hyphae was taken out and placed to the flask containing MEB. The flasks were then kept at 30 °C in an incubator. Following that, 50 ml of ethyl acetate will be added to each flask, and they will once more be shaken at 150 revolutions per minute at 45 °C. The supernatant is taken out of each flask after two to six hours. The Rota evaporator was used to dry the extracted supernatant in order to get a concentrated organic phase.

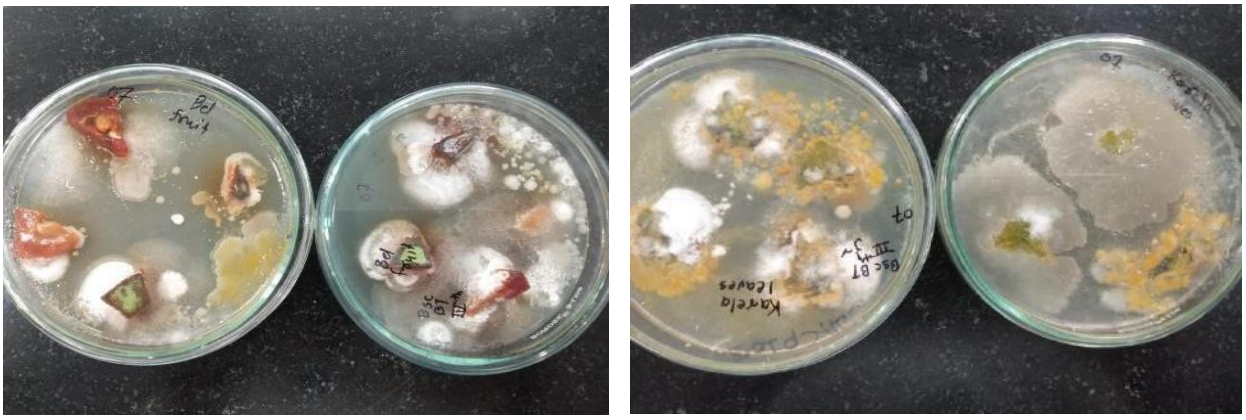
- **Antidiabetic Assays:** The alpha-amylase inhibitory assay is the most often used method for this purpose. The procedure involves adding 200 µL of fungal extract (15 ml) to 200 µL of sodium phosphate buffer (0.02M) with a pH of 6.7 and 0.5 mg/ml alpha-amylase solution. After 10 minutes of incubation at 30°C, 200 µL of a starch (1%) solution is added to the tubes containing the combination. For ten minutes, the reaction mixtures are once more incubated at 30°C. The reaction mixture is now stopped by adding 500 µL of DNS (3, 5-dinitrosalicylic acid) reagent. After five minutes of incubation at 92°C in a pre-made water bath, the mixes in tubes will be allowed to cool to 40°C. After cooling the mixture at room temperature, 6 ml of sterilized deionized water was added to each tube. Absorbance will be measured at 540 nm using a spectrophotometer. The inhibitory activity of alpha-amylase was measured using the formula:

$$\% \text{ Inhibition} = \left[\frac{(A_c - A_e)}{A_c}\right] \times 100$$

Where, A_c: Absorbance of control
A_e: Absorbance of extract.

- **RESULT / FINDINGS:**

1. Isolation of Endophytic Fungi



(a) (b)

Fig. 1(a) & (b). Fungi growth on the PD agar medium

Endophytic fungi were successfully isolated from healthy leaf and fruit samples of *Aegle marmelos* and *Momordica charantia*. After surface sterilization, the plant tissue segments were cultured on malt extract agar (MEA) plates supplemented with streptomycin sulphate to inhibit bacterial growth. Visible fungal growth was observed within 3–4 days of incubation at 28°C ± 2°C. Four distinct fungal colonies were isolated, purified, and maintained on PDA slants for further analysis. Each isolate displayed unique macroscopic features, including differences in colony morphology, colour, texture, and growth pattern. The four isolated species were *Fusarium oxysporum*, *Aspergillus versicolor*, *Aspergillus niger and Penicillium verhagenii.*

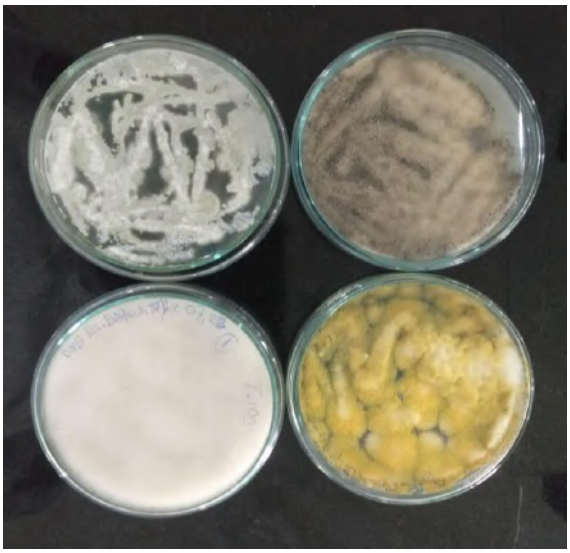


Fig. 2. Fungi cultured on agar medium

2. Morphological Characterization of Isolated Fungi

The isolated fungi were characterized morphologically using lactophenol cotton blue staining to study their microscopic structures. Observations revealed distinct hyphal arrangements, branching patterns, and sporulation features. These morphological traits were instrumental in identifying the fungi as *Fusarium oxysporum*, *Aspergillus versicolor*, *Aspergillus niger*, and *Penicillium verhagenii*. A detailed summary of their macroscopic and microscopic characteristics is provided in **Table 1** and the Macro & Microscopic view of Fungal Extracts can be seen in **Table 4**.

Table 1: Morphological Characteristics of Isolated Fungi

Fungal Species	Macroscopic Features	Microscopic Features	Form	Elevation	Margin
Fusarium oxysporum	Cottony white colony	Hyphae with head-tail-like structure	Filamentous	Raised	Lobate
Aspergillus versicolor	Greenish colony with radial grooves	Cylindrical structures	Irregular	Flat	Filiform
Aspergillus niger	Black, powdery colonies	Hyphae with long tail-like structure and a head	Filamentous	Umbonate	Entire
Penicillium verhagenii	Bluish-green colony	Central bulb surrounded by thread-like appendages	Filamentous	Raised	Entire

3. Production of anti-diabetic metabolites from endophytes

Visible fungal biomass developed within 4–5 days, varying by fungal species. After a 10-day incubation period, an equal volume (50 mL) of ethyl acetate was added to the culture for metabolite extraction. The flasks were shaken for 2–6 hours at 45°C, after which the organic phase containing the metabolites was separated and concentrated using rotary evaporation. The resulting extract was stored at 4°C for further antidiabetic assays.

Distinct differences in metabolite characteristics, such as color, viscosity, and volume, were noted among the isolates, reflecting their unique metabolic profiles. *Fusarium oxysporum* exhibited the highest yield and viscosity, indicating a potentially higher concentration of bioactive compounds.

Table 2: Observations During the Production of Metabolites

Fungal Species	Biomass Growth (Days)	Metabolite Appearance	Volume of Organic Phase (mL)
Fusarium oxysporum	4–5	Pale yellow, viscous	4.5
Aspergillus versicolor	3–4	Greenish-brown, fluid	3.8
Aspergillus niger	4	Dark brown, semi-viscous	4.0
Penicillium verhagenii	5	Light blue, watery	3.5

The antidiabetic activity of the fungal extracts was evaluated through the α-amylase inhibitory assay. This assay measures the ability of fungal metabolites to inhibit α-amylase, an enzyme critical for breaking down starch into glucose. By reducing the activity of α-amylase, these extracts have the potential to lower postprandial blood glucose levels, which is a key therapeutic goal in diabetes management.

4. α-Amylase Inhibition

The fungal extracts exhibited varying levels of α-amylase inhibition at a concentration of 200 µg/mL. Among the isolates, *Fusarium oxysporum* demonstrated the highest inhibitory activity, followed by *Aspergillus niger*, *Aspergillus versicolor*, and *Penicillium verhagenii*. These results indicate that metabolites of *Fusarium oxysporum* contain bioactive compounds with a strong potential for regulating carbohydrate metabolism. The control sample (without fungal extract) showed no inhibition, confirming that the observed effects were solely due to the fungal metabolites.The high α-amylase inhibitory activity observed in *Fusarium oxysporum* suggests that it is a promising source of bioactive antidiabetic compounds. The moderate inhibitory activities of *Aspergillus niger* and *Aspergillus versicolor* also highlight their potential, albeit at a lower efficacy compared to *Fusarium oxysporum*. On the other hand, *Penicillium verhagenii* exhibited the lowest inhibitory activity, indicating that its metabolite composition may be less effective for α-amylase inhibition under the tested conditions.

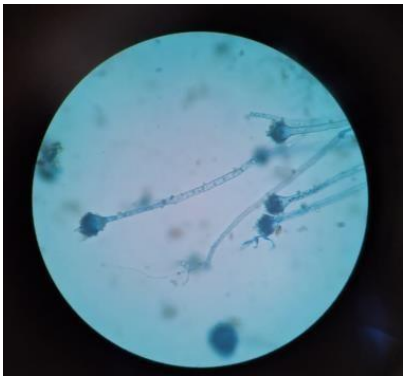
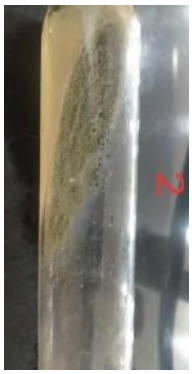
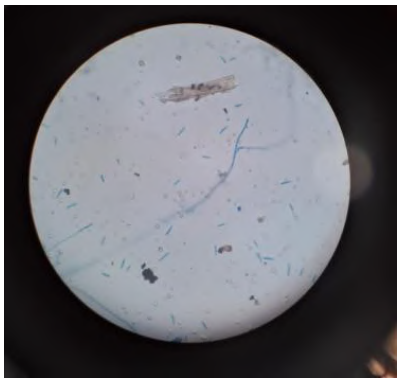
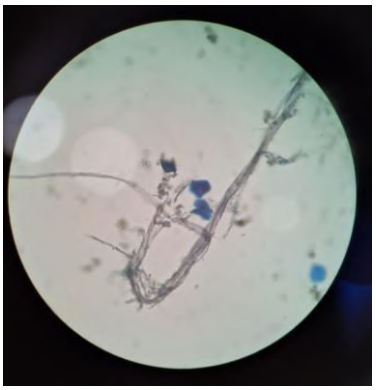
Statistical analysis revealed significant differences in the α-amylase inhibition percentages among the fungal isolates ($p < 0.05$). This confirms the variability in the bioactive potential of metabolites produced by different fungi. Further studies could optimize extraction protocols or adjust fungal culture conditions to enhance metabolite efficacy. However, the inhibition of α-amylase by fungal metabolites could be attributed to specific bioactive compounds, such as phenolics, flavonoids, or alkaloids, known for their enzyme-inhibitory properties. These compounds may interfere with the active site of α-amylase, reducing its ability to hydrolyze starch into glucose. Future research involving chemical characterization of the fungal extracts is essential to identify the active components responsible for this activity.

Table 3: α-Amylase Inhibitory Activity of Fungal Extracts

Sample	Optical Density at 540 nm (OD)	Percentage Inhibition (%)
Control	0.52	0.0
Fusarium oxysporum	0.24	53.8
Aspergillus versicolor	0.36	30.7
Aspergillus niger	0.33	36.5
Penicillium verhagenii	0.40	19.2

Fig. 3. MEB broth containing Fungi culture on agar

Table 4: Macro and Micro view of Fungal Extracts

Sr. No	Fungal Spp.	Macro view of fungus	Micro view of fungus
1.	*Fusarium oxysporum*		
2.	*Aspergillus versicolor*		
3.	*Aspergillus niger*		
4.	*Penicillium verhagenii*		

6. DISCUSSION :

The present study investigated the antidiabetic potential of endophytic fungi isolated from *Aegle marmelos* and *Momordica charantia*. The findings revealed that these fungi produce secondary metabolites capable of inhibiting α-amylase, a key enzyme involved in carbohydrate metabolism. Among the four isolates, *Fusarium oxysporum* exhibited the highest inhibitory activity (53.8%), suggesting its metabolites are the most potent in regulating postprandial glucose levels. This aligns with existing studies that highlight the bioactive potential of endophytic fungi in producing enzyme inhibitors.

The moderate inhibitory activities observed in *Aspergillus niger* (36.5%) and *Aspergillus versicolor* (30.7%) suggest that these fungi also produce metabolites with antidiabetic properties, albeit less potent than *Fusarium oxysporum*. On the other hand, the lower activity of *Penicillium verhagenii* (19.2%) indicates variability in the metabolic profiles and bioactive compound production among endophytic fungi.

The differences in α-amylase inhibition percentages can be attributed to the unique metabolic pathways of each fungal species, which govern the synthesis of secondary metabolites. Factors such as the composition of the growth medium, incubation conditions, and extraction protocols may also influence the yield and activity of these compounds. The presence of specific bioactive compounds, such as phenolics and flavonoids, known for their enzyme-inhibitory properties, may explain the observed activity. However, detailed chemical characterization is required to identify the exact compounds responsible for α-amylase inhibition.

This study underscores the significance of exploring endophytic fungi as a promising source of natural antidiabetic agents. While the results are encouraging, optimizing culture conditions, scaling up metabolite production, and performing in vivo studies will be essential for validating the therapeutic potential of these fungal extracts.

• CONCLUSION / SUMMARY:

This study demonstrates that endophytic fungi isolated from *Aegle marmelos* and *Momordica charantia* possess significant antidiabetic potential through their α-amylase inhibitory activity. Among the isolates, *Fusarium oxysporum* emerged as the most promising candidate, showing the highest enzyme inhibition. These findings highlight the potential of endophytic fungi as a sustainable source of natural bioactive compounds for diabetes management.

The results provide a strong foundation for future research aimed at optimizing metabolite production and exploring the mechanisms underlying the antidiabetic effects of fungal extracts. Additionally, the identification of specific bioactive compounds and their clinical validation could pave the way for the development of novel, cost-effective therapeutics for diabetes treatment. This study reinforces the importance of bioprospecting fungal endophytes as valuable contributors to natural product-based drug discovery.

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