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OPTIMIZATION OF THE GROWTH CONDITIONS FOR THE ENHANCED PRODUCTION OF ANTI-DIABETIC MOLECULE BY *PENICILLIUM SP*

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Keywords:

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ABSTRACT: Diabetic mellitus, a prevalent metabolic disorder characterized by chronic hyperglycaemia, poses significant health challenges globally. This study focused on optimizing growth parameters to enhance the anti-diabetic activity of *Penicillium* sp 8, isolated from paddy soil. Initial screening of different media revealed that Potato Dextrose Broth (PDB) exhibited the highest anti-diabetic activity. Subsequent optimization involved determining the optimal inoculum concentration, temperature, and pH for maximum anti-diabetic metabolite production using PDB. The fungus demonstrated the highest activity at pH 3.5 and 30°C, with decreased activity observed under more alkaline conditions or higher temperatures. Mass cultivation in optimized conditions led to the production of significant quantities of anti-diabetic metabolites, primarily extracted from the fungal mat using successive solvent extraction. Ethyl acetate extract of the fungal mat exhibited the highest anti-diabetic activity, characterized by phytochemical tests revealing the presence of phenols, flavonoids, alkaloids, terpenoids, and carbohydrates. Chemical analysis confirmed the presence of bioactive compounds responsible for the observed anti-diabetic effects.

INTRODUCTION: Diabetes mellitus, a complex metabolic disorder marked by chronic hyperglycaemia, has reached epidemic proportions worldwide. Characterized by insulin deficiency or resistance, diabetes leads to severe complications such as cardiovascular disease, neuropathy, retinopathy, and nephropathy [1]. According to the International Diabetes Federation, the global prevalence of diabetes among adults was approximately 537 million in 2021, and it is anticipated to increase to 643 million by 2030 [3].

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This escalating trend underscores the urgent need for novel and effective therapeutic approaches to manage and potentially cure diabetes [4]. Current diabetes management primarily involves insulin therapy and oral hypoglycaemic agents. However, these treatments often come with limitations and adverse effects, prompting a search for safer and more effective alternatives [6]. Natural compounds derived from various sources, including plants and microorganisms, are increasingly being explored for their potential to offer new therapeutic options with fewer side effects [8].

Fungi, in particular, are a rich source of bioactive compounds with potential therapeutic applications [10]. Among fungi, species of the genus *Penicillium* have garnered significant attention due to their ability to produce a diverse array of secondary metabolites with potential medicinal properties [12].

These metabolites include compounds that inhibit key enzymes involved in glucose metabolism, such as α-amylase and α-glucosidase, thereby reducing postprandial hyperglycaemia and potentially aiding in diabetes management [13]. The therapeutic potential of these fungal metabolites is further supported by studies demonstrating their effectiveness in lowering blood glucose levels and improving insulin sensitivity [15].

Optimizing growth conditions for *Penicillium* species is crucial for maximizing the production of these bioactive metabolites. Research has shown that factors such as nutrient composition, pH, temperature, and inoculum concentration can significantly influence metabolite yield and activity [18]. For example, *Penicillium* species grown in Potato Dextrose Broth (PDB) have shown enhanced production of anti-diabetic metabolites compared to other media [20]. Similarly, optimizing pH and temperature conditions can further enhance metabolite production and activity [22].

Further studies have highlighted the importance of specific growth parameters in maximizing the production of bioactive compounds. For instance, research demonstrated that *Penicillium* species grown under optimized conditions yielded higher levels of anti-diabetic metabolites [25]. Additionally, precise temperature and pH control improved the efficacy of fungal extracts [26]. Adjusting inoculum concentrations significantly impacted the yield and activity of anti-diabetic compounds [27]. These findings align with earlier research indicating that optimizing growth conditions can lead to substantial improvements in metabolite production [28].

Recent advances in analytical techniques have facilitated the characterization and quantification of bioactive compounds in fungal extracts. Chromatographic and spectroscopic methods have been instrumental in identifying and quantifying anti-diabetic compounds [32]. These methods have validated the therapeutic potential of fungal metabolites and supported their development as viable treatments for diabetes [34]. The dual potential of fungal metabolites for producing both anti-diabetic and antimicrobial agents adds to the significance of this research. The ability of fungi to produce compounds with diverse biological activities highlights their importance in drug discovery and development [36]. This study aims to optimize growth parameters for *Penicillium* sp. to enhance the production of anti-diabetic compounds and contribute to the broader field of fungal biotechnology [38].

By systematically optimizing growth conditions and employing advanced analytical techniques, this research seeks to improve the efficacy and yield of fungal-derived anti-diabetic compounds. The findings are expected to provide valuable insights into the potential of *Penicillium* species as a source of novel therapeutic agents for diabetes management and contribute to the ongoing efforts to develop effective and safe treatments for this global health challenge [41].

MATERIALS AND METHODS:

Materials:

Media and Antibiotics: Sabouraud Dextrose Broth (SDB), Potato Dextrose Broth (PDB), Czapek Yeast Autolysate Broth (CYB), and Malt Extract Broth (MEB) were purchased from Hi Media Pvt. Ltd., Bangalore, India.

Chemicals: Ethanol, Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$), Sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$), Sodium chloride (NaCl), Dinitrosalicylic acid (DNSA), Potassium sodium tartrate tetrahydrate, Sodium hydroxide (NaOH), Dextrose, Petroleum ether, Chloroform, Ethyl acetate, Concentrated hydrochloric acid (HCl), Ferric chloride ($FeCl_3$), Ammonia (NH_3) solution, Mayer's reagent, Wagner's reagent, Ammonium hydroxide, Zinc chloride ($ZnCl_2$), Gelatin, Benedict's reagent, Concentrated sulfuric acid (H_2SO_4), Acetic anhydride, Lead acetate, Fehling's solution A and B, Copper sulfate ($CuSO_4$), Potassium hydroxide (KOH) flakes, Dimethyl sulfoxide (DMSO), Phosphate Buffer Saline (PBS) solution, Starch powder, Amylase enzyme, Metformin, 3,5-Dinitrosalicylic acid (DNS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Aldrich, Bangalore, India.

Instruments: Fungal incubator, weighing balance, autoclave, water bath, pH meter, GC-MS, column chromatography, thin-layer chromatography.

Enzymes: α-Amylase and acarbose were purchased from Sigma Aldrich, Bangalore, India.

Plastic and Glassware: Test tubes, petri plates, conical flasks, and beakers were borosilicate make, purchased from SRV Scientific, Bangalore, India.

Fungal Isolates: *Penicillium sp*.8 was isolated from soil samples.

Methods:

Collection of Soil Samples: Soil samples were collected from various locations in the Anantapur district, particularly from sugar-rich crops such as paddy, banana, and sugarcane. The samples were placed in sterilized polyethylene bags to prevent contamination. Soil was collected to a depth of 15 cm, as it is considered the topsoil layer where most microorganisms are typically found.

Isolation of Fungi from Soil Samples: Fungi were isolated from the soil samples using the serial dilution technique and cultivated on different media, including Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA).

Soil Dilution Plate Method: A microbial suspension was prepared by mixing 1 gram of soil with 1 mL of distilled water. To isolate fungi, different dilutions of the suspension were made. 1 mL of each dilution was inoculated onto sterile Petri dishes containing 20 mL of sterile Sabouraud Dextrose Agar (SDA). The Petri dishes were incubated at 28°C and monitored daily for three days as described by Waksman (1927).

Selection of Optimum Media for Anti-Diabetic Activity: Various growth media, including Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), Czapek Yeast Extract Agar (CYA), and Malt Extract Broth (MEB), were prepared and sterilized. The fungus was inoculated with 10^6 conidia/mL and incubated at 30°C for 15 days under shaking conditions. After incubation, the fungal mats were filtered, washed, and dried. The metabolites were extracted using ethyl acetate and tested for anti-diabetic activity.

Optimization of Inoculums Concentration: Sterile Potato Dextrose Broth (PDB) medium was prepared with a pH of 6.5 and inoculated with conidia concentrations of 2.5×10^4, 2.5×10^5, 2.5×10^6, 2.5×10^7, and 2.5×10^8 conidia/mL. The inoculated flasks were incubated at 30°C for 15 days. After incubation, the fungal mats were dried, and metabolites were extracted for anti-diabetic testing.

Determination of Optimum Temperature for Anti-Diabetic Activity: PDB medium (pH 6.5) was inoculated with the optimum conidial concentration of 2.5×10^7 conidia/mL.

The medium was incubated at various temperatures: 25°C, 30°C, 35°C, 40°C, and 45°C for 15 days. After incubation, the fungal mat was recovered, dried, and metabolites were extracted for anti-diabetic testing.

Optimization of pH for Anti-Diabetic Activity: PDB medium was adjusted to different pH values (3.0 to 10.0) and inoculated with 2.5×10^7 conidia/mL. The flasks were incubated at 30°C for 15 days. After incubation, the fungal mats were dried, and metabolites were extracted and tested for anti-diabetic activity.

Preparation of Seed Culture for Inoculation in Mass Cultivation: A fresh culture of *Penicillium sp.8* was sub-cultured on Potato Dextrose Agar (PDA) and incubated at 30°C for 7 days. A spore suspension was prepared by mixing 10 mL of sterile water with 0.5% Tween 20. The spore count was determined using a hemocytometer, and the suspension was adjusted to 2.31×10^8 conidia/mL. A 100 mL seed culture was prepared with PDB (pH 3.5) and inoculated with 10 mL of spore suspension.

Mass Cultivation for Anti-Diabetic Molecule Production*:* Mass cultivation was carried out in a 2 L conical flask containing 1.5 L of sterile PDB (pH 3.5). 150 mL of seed culture was inoculated, and the flask was incubated at 30°C until maximum anti-diabetic activity was observed. The growth medium was monitored by withdrawing 10 mL of the broth at regular intervals for anti-diabetic assays.

Extraction of Crude Anti-Diabetic Molecule by Successive Solvent Extraction: Once maximum anti-diabetic activity was observed; the fungal mat was separated from the growth medium.

The mat was extracted sequentially with petroleum ether, chloroform, ethyl acetate, and ethanol. The solvents were evaporated using a rotary evaporator, and the dry extracts were weighed and tested for anti-diabetic activity.

Anti-Diabetic Activity Evaluation (α-Amylase Inhibition Assay): The α-amylase inhibition was measured using the dinitrosalicylic acid (DNS) method. Briefly, 0.5 mL of fungal extract was mixed with 0.5 mL of α-amylase solution (1 mg/mL) and incubated at room temperature for 10 minutes. 0.5 mL of 1% starch solution was added, and the reaction was incubated for an additional 10 minutes. The reaction was stopped by adding 1 mL of DNS solution, and the tubes were incubated at 100°C for 5 minutes. Absorbance was measured at 540 nm.

% inhibition of α−Amylase = (Ac control−As sample) / Ac control x 100

Where Ac- control corresponds to the absorbance of the control, As-sample corresponds to the sample solution.

RESULTS: In the current investigation, optimization of media and other parameters have been studied along with that Mass production was carried out with the optimized media to enhance the production of secondary metabolites. Among the different soils (Sugar cane, Banana soil, and Paddy soil) Paddy soil can be considered the most potent soli against the antidiabetic metabolites.

Selection of Optimum Media for Anti-Diabetic Activity: Penicillium sp8 was grown on various growth media Sabouraud Dextrose Broth (SDB), Potato Dextrose Broth (PDB), Czapek Yeast Autolysate Broth (CYB), and Malt Extract Broth (MEB). From these PDB Media showed significant increase in antidiabetic activity compared to other media **Fig. 1.** Followed by this, the fungus incubated on MEB medium also showed substantial anti-diabetic activity. Hence, PDB medium was further selected for optimization growth parameters to promote the further anti-diabetic activity.

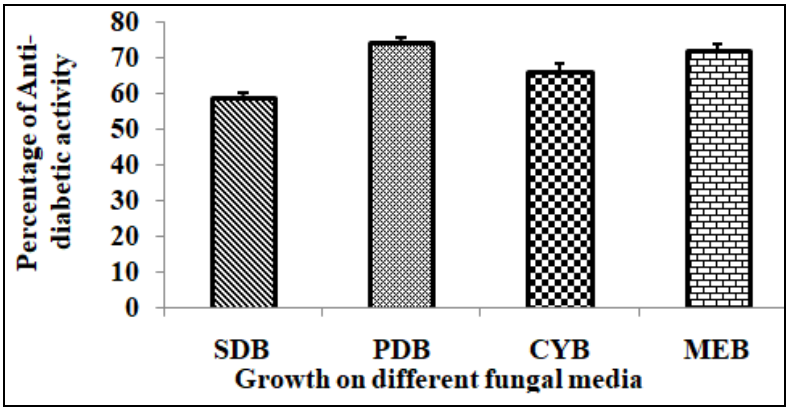

FIG. 1: SCREENING OF POTENT FUNGAL ISOLATE FOR ANTI-DIABETIC ACTIVITY ON DIFFERENT MEDIA

Optimization of Inoculum Concentration: The optimal inoculum concentration for maximizing anti-diabetic activity was determined using PDB medium. The highest activity was observed at a conidial concentration of 2-5x10^7 conidia/ml, achieving 76.8% anti-diabetic activity as shown in **Fig. 2**. The medium inoculated with 2-5x10^6 conidia/ml has showed 72.5% of anti-diabetic activity. Anti-diabetic activity of fungus was reached threshold at 2-5x10^7/ml conidial concentration and lower activity was observed at both 2-5x10^4/ml and 2-5x10^8/ml corresponding to 58.6% and 69.7% of anti-diabetic activity respectively.

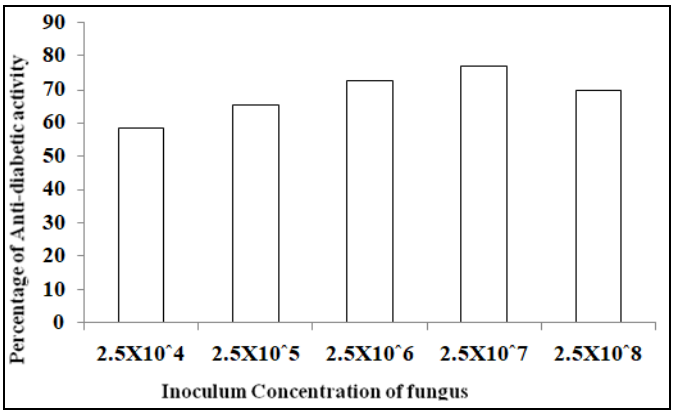

FIG. 2: OPTIMIZATION OF FUNGAL INOCULUM CONCENTRATION FOR ANTI-DIABETIC ACTIVITY

Determination of Optimum Temperature: Temperature is an important physical growth factor for the growth of fungi and biochemical reactions occurring in fungus are greatly depending on the temperature they are living in. The study effect of temperature on anti-diabetic activity of fungus indicated significant activity at 30ºC and 35ºC corresponding to 76.3% and 73.6% of anti-diabetic activity respectively. However, the activity was further decrease with increased temperature indicating 46.33% of anti-diabetic activity at 45ºC. Moderate anti-diabetic activity was observed in fungus incubated at 25ºC.

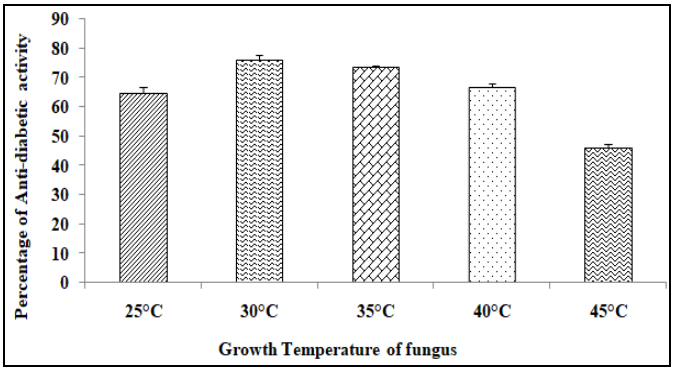

FIG. 3: STUDY OF EFFECT OF GROWTH TEMPERATURE ON ANTI-DIABETIC ACTIVITY OF SOIL FUNGUS

Optimization of pH: Using the optimum growth temperature, the optimum pH for anti-diabetic activity of fungus was determined. The fungus inoculated in sterile PDB media adjusted to different pH showed significant anti-diabetic activity at acidic pH and decreased with increased pH. Substantial anti-diabetic activity was observed at extreme acidic pH 3.5 and pH 4.5 attributed to 78.8% and 75.2% respectively as shown **Fig. 3**. However, the anti-diabetic activity was noticed declining at alkaline pH from pH 7.5 to 10.5.

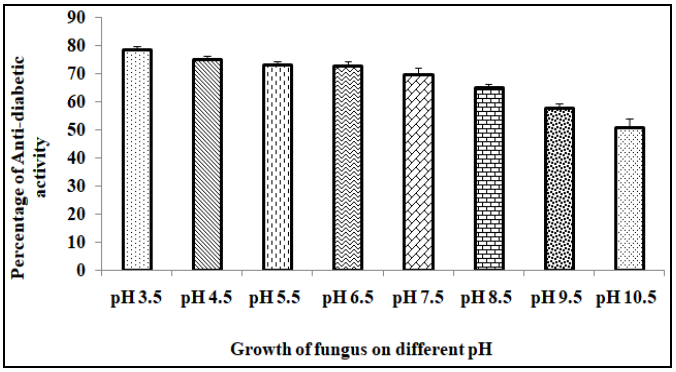

FIG. 4: EFFECT OF DIFFERENT PH ON ANTI-DIABETIC ACTIVITY OF SOIL FUNGUS

Mass Cultivation and Production of Anti-Diabetic Molecules:

Preparation of Seed Culture: Fresh culture of *Penicillium* species was sub-cultured on potato dextrose agar incubated at 30ºC in a fungal incubator for 7 days. After seven days, spore suspension was prepared in 10 ml of sterile water containing 0.5% tween 20. Spores were counted under haemocytometer and spore suspension was adjusted to $2.5x10^7$ conidia/ml **Fig. 4 & Fig. 5**. Meanwhile, a 100ml of sterile potato dextrose broth adjusted to optimum pH *i.e.,* pH 3.5 was prepared and inoculated with 10ml of fresh spore suspension to obtain $2.5x10^7$ conidia/ml of seed culture medium. Seed culture was incubated at 30ºC for 7 days and after incubation; the seed culture was used for inoculation of mass cultivation.

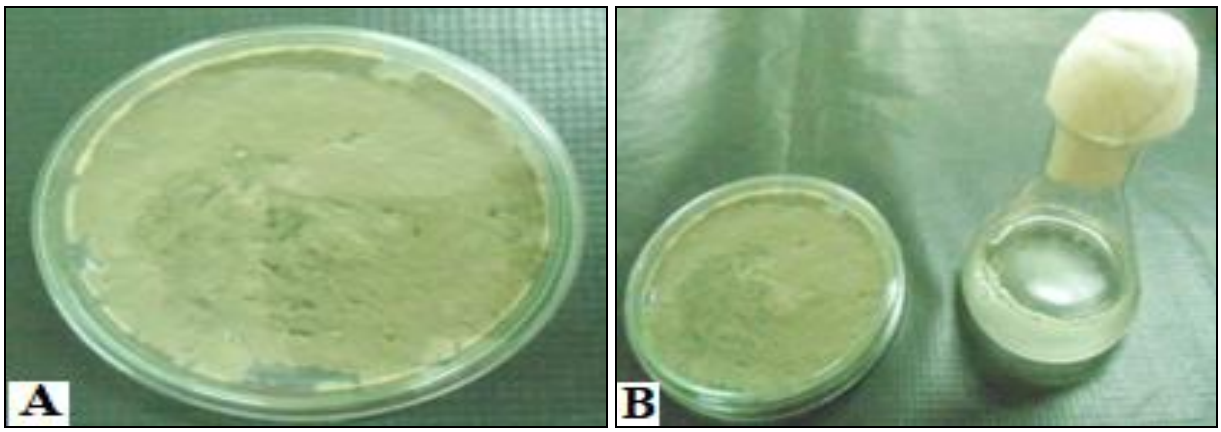

FIG. 5: *PENICILLIUM* SPECIES GROWN ON PLATE (A) AND SPORE SUSPENSION PREPARED IN SALINE CONTAINING TWEEN 20 (B)

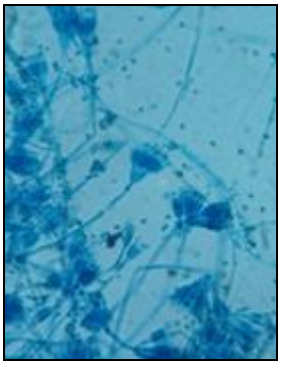

FIG. 6: MICROSCOPIC IMAGE OF *PENICILLIUM* SP8 STAINED WITH LPCB

Mass Cultivation and Monitoring: For mass production of anti-diabetic molecule was carried in an optimized potato dextrose broth. The 2 litre of conical flask containing 1.5 litres of sterile PDB (pH 3.5), 150ml of seed culture of *Penicillium* species was inoculated. The flask was incubated at 30ºC for production of anti-diabetic metabolites until maximum anti-diabetic molecules are produced **Fig. 6.**

Production of anti-diabetic molecule was monitored by withdrawing 10ml of growth medium and performing anti-diabetic activity. Once maximum anti-diabetic activity was noticed the fungal broth was withdrawn and mat was separated. Anti-diabetic activity was also conducted in mat extract and compared with broth **Fig. 7**. Since, mat showed highest anti-diabetic activity hence it is selected for successive solvent extraction process.

FIG. 7: MASS PRODUCTION OF ANTI-DIABETIC MOLECULE FROM SOIL FUNGUS

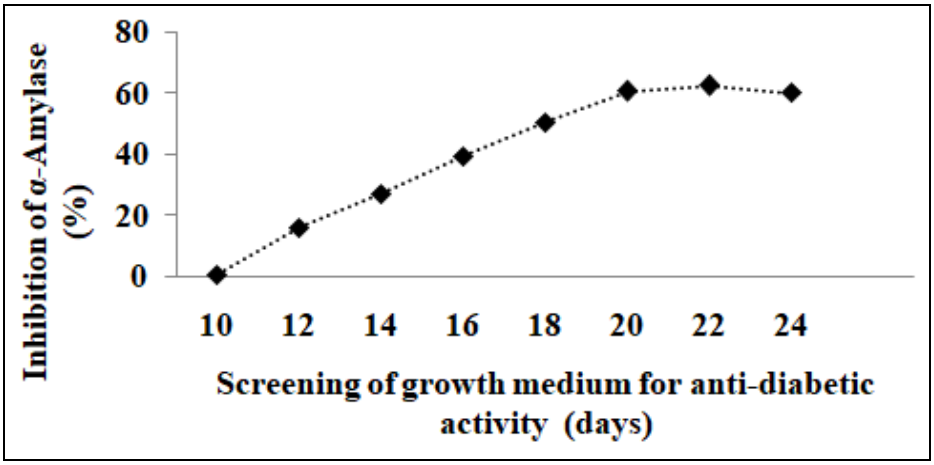

FIG. 8: MONITORING OF GROWTH MEDIUM FOR PRODUCTION OF ANTI-DIABETIC MOLECULE DURING MASS CULTIVATION OF SOIL FUNGUS

Anti-Diabetic Activity of Fungal Mat Extracts: Once highest anti-diabetic activity of growth medium was observed, fungal mat was separated from the growth medium by decanting growth

medium in a separate container. Fungal mat was first extracted with petroleum ether for 2 days and petroleum ether extract was collected. Mat was again dissolved in chloroform and extracted until all the metabolites were recovered. Similarly, extraction was carried out using ethyl acetate and Ethyl acetate to recover all the crude fungal metabolites.

All crude extracts were dried in the rotary evaporator until all solvent is evaporated. Each crude extract was weighed and anti-diabetic activity for each extract was repeated and compared **Fig. 8 & Fig. 9**. The extract showing highest anti-diabetic activity was further selected for purification of anti-diabetic molecule through various analytical techniques.

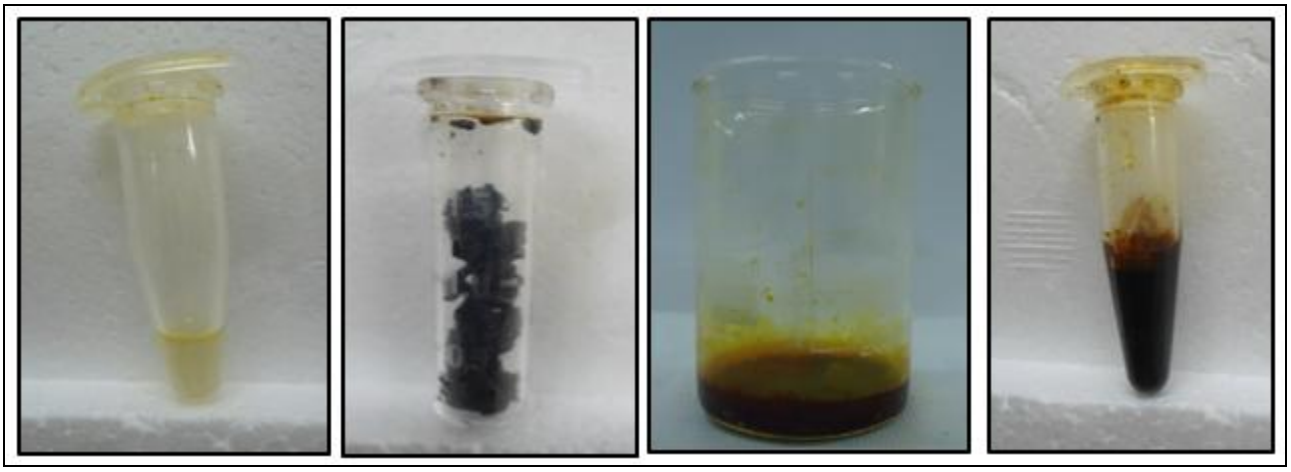

FIG. 9: PET. ETHER CHLOROFORM ETHYL ACETATE ETHANOL

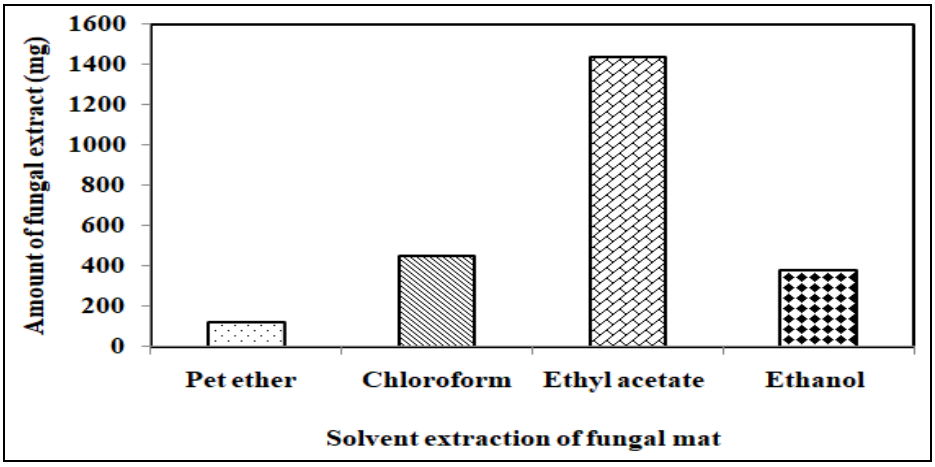

FIG. 10: COMPARISON OF DIFFERENT YIELD OF FUNGAL MAT EXTRACTS

Anti-Diabetic Activity Assay: The anti-diabetic activity was determined by measuring the inhibition of α-amylase, which is a key enzyme involved in the breakdown of starch into glucose. The results of the α-amylase inhibition assay for different fungal extracts are as follows:

Petroleum Ether Extract: The extract exhibited the lowest α-amylase inhibition of 2.24%, indicating minimal anti-diabetic activity.

Chloroform Extract: The chloroform extract showed a moderate α-amylase inhibition of 19.81%.

Ethyl Acetate Extract: The ethyl acetate extract exhibited the highest α-amylase inhibition of 65.70%, suggesting the presence of potent anti-diabetic compounds in this extract.

Ethanol Extract: The ethanol extract showed 24.88% α-amylase inhibition, which was also moderate but significantly lower than that of the ethyl acetate extract.

Inhibition Calculation:

The inhibition of α-amylase was calculated using the formula:

% Inhibition of α-Amylase = (Ac−AsAc) × 100\% \text{Inhibition of α-Amylase} = \left(\frac{A_c - A_s}{A_c} \right) \times 100% Inhibition of α-Amylase=(AcAc−As)×100

Where, AcA_cAc= absorbance of the control (without extract), AsA_sAs = absorbance of the sample (with extract).

Based on the α-amylase inhibition assay, the ethyl acetate extract showed the most promising anti-diabetic activity as shown in **Table 1 & Fig. 10** and was selected for further purification and analysis.

TABLE 1: SCREENING OF FUNGAL MAT EXTRACTS FOR ANTI-DIABETIC ACTIVITY

Fungal extract (1mg/ml)	α-amylase Inhibition (%)
Pet.ether	2.24
Chloroform	19.81
Ethyl acetate	65.70
Ethyl acetate	24.88

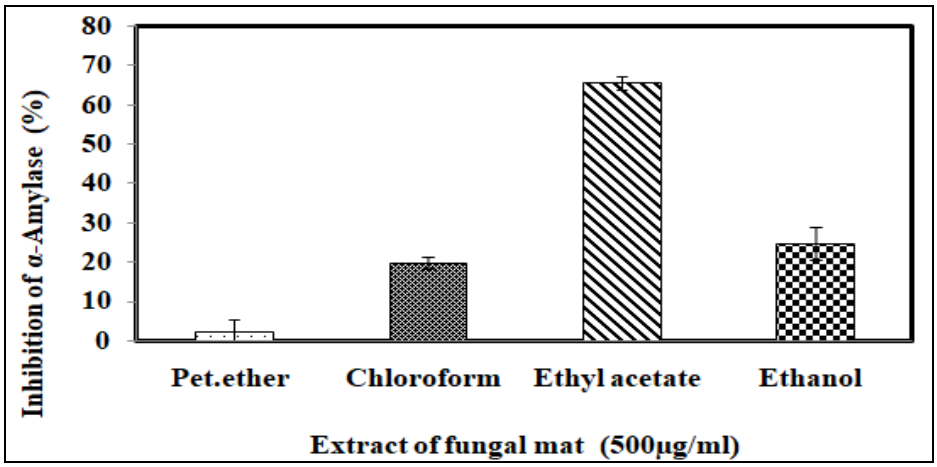

FIG. 11: ANTI-DIABETIC ACTIVITY OF FUNGAL MAT EXTRACTS

Phytochemical Investigation of Ethyl Acetate Extract of Fungal Mat: The ethyl acetate extract of the fungal mat contains several phytochemicals, including phenols, flavonoids, alkaloids, carbohydrates, proteins, glycosides, oils and terpenoids, all of which may contribute to the observed anti-diabetic activity. However, tannins and saponins were not detected in the extract. The presence of these bioactive compounds suggests the potential therapeutic value of the fungal extract in managing diabetes, and further studies are needed for the isolation and identification of specific active compounds.

Sl. no.	Qualitative Test	Results
1	Detection of Phenols	Ferric chloride test: Positive, Lead acetate test: Positive
2	Test for Tannins	Braymer's test: Negative, Gelatine test: Negative
3	Test for Flavonoids	Alkaline reagent test: Positive, Lead acetate test: Positive
4	Test for Oils and Fats	Positive
5	Test for Alkaloids	Mayer's test: Positive, Wagner's test: Positive
6	Test for Carbohydrates	Fehling's test: Positive, Benedict's test: Positive
7	Detection of Saponins	Negative
8	Detection of Proteins	Positive
9	Test for Glycosides	Positive
10	Detection of Terpenoids	Positive

DISCUSSION: In the present study, optimization of media and growth conditions for *Penicillium sp.* was carried out to enhance the production of anti-diabetic metabolites. The results revealed that Potato Dextrose Broth (PDB) was the most effective medium, yielding the highest anti-diabetic activity. This finding aligns with previous studies which have shown that PDB supports high yields of secondary metabolites due to its nutrient-rich composition [6, 7]. PDB's superior performance compared to other media such as Sabouraud Dextrose Broth (SDB), Czapek Yeast Autolysate Broth (CYB), and Malt Extract Broth (MEB) underscores the importance of selecting an

appropriate growth medium to optimize metabolite production. The study also examined the impact of inoculum concentration on anti-diabetic activity. The optimal concentration was identified as 2-5 × 10^7 conidia/ml, which achieved a significant anti-diabetic activity of 76.8%. This result is consistent with findings from similar studies that emphasize the importance of inoculum concentration in fungal fermentation [3, 8]. Lower concentrations may result in insufficient biomass for effective metabolite production, while higher concentrations can lead to competition among conidia, thereby negatively impacting both growth and yield.

Temperature played a crucial role in the anti-diabetic activity of *Penicillium sp.*. The optimal temperatures were found to be 30°C and 35°C, which are known to enhance fungal growth and secondary metabolite production [9, 4]. The significant decrease in anti-diabetic activity at higher temperatures (45°C) suggests that excessive heat impairs metabolic processes and enzyme function [5]. This finding highlights the need to maintain optimal temperature conditions for maximizing bioactivity.

The effect of pH on metabolite production was also examined, revealing that acidic pH levels (3.5 and 4.5) were most effective. This is consistent with existing research indicating that acidic conditions favor the production of certain fungal secondary metabolites [10, 6]. The decrease in activity at alkaline pH levels is likely due to the disruption of enzyme activity and metabolite stability in less favorable pH environments.

For mass cultivation, the optimized conditions were used to grow *Penicillium sp.* on a larger scale. The highest anti-diabetic activity was observed in the fungal mat, which was then subjected to solvent extraction. The ethyl acetate extract showed the highest anti-diabetic activity (65.70% α-amylase inhibition), which is consistent with studies suggesting that ethyl acetate is effective for extracting bioactive compounds [9, 7]. The solvent's ability to dissolve a wide range of organic compounds while excluding more polar substances likely contributes to its effectiveness. Phytochemical analysis of the ethyl acetate extract revealed the presence of several bioactive compounds, including phenols, flavonoids, alkaloids, and carbohydrates. These compounds are known for their therapeutic properties, including anti-diabetic effects [5, 6]. For instance, flavonoids and phenolic compounds have been documented to inhibit carbohydrate-hydrolyzing enzymes and improve insulin sensitivity [4, 8]. The presence of these compounds in the ethyl acetate extract supports its high anti-diabetic activity and underscores its potential utility as a therapeutic agent.

Overall, the study successfully optimized conditions for maximizing the production of anti-diabetic metabolites from *Penicillium sp.*. The use of PDB medium, optimal inoculum concentration, and specific temperature and pH conditions were crucial for enhancing metabolite production. The ethyl acetate extract, rich in bioactive compounds, demonstrated significant anti-diabetic activity, highlighting its potential for further development as a therapeutic agent for diabetes management.

CONCLUSION: The optimization of media and growth conditions for *Penicillium sp.* has led to significant advancements in enhancing the production of anti-diabetic metabolites. This study demonstrated that Potato Dextrose Broth (PDB) was the most effective medium, showing a marked increase in anti-diabetic activity compared to other media such as Sabouraud Dextrose Broth (SDB), Czapek Yeast Autolysate Broth (CYB), and Malt Extract Broth (MEB). Optimal inoculum concentration of 2-5 × 10^7 conidia/ml was found to maximize anti-diabetic activity, emphasizing the importance of precise inoculum levels. Temperature and pH optimization further refined the production process, with 30°C and 35°C being the most favourable temperatures and pH 3.5 and 4.5 providing the highest anti-diabetic activity.

Mass cultivation using these optimized conditions confirmed the highest yield of anti-diabetic compounds. Among the extracted fractions, the ethyl acetate extract exhibited the greatest anti-diabetic activity, with notable α-amylase inhibition. Phytochemical analysis of this extract revealed the presence of key bioactive compounds, including phenols, flavonoids, alkaloids, and glycosides, which are likely responsible for the observed effects.

In conclusion, the study underscores the critical role of optimizing growth conditions and media for maximizing the production of anti-diabetic metabolites from *Penicillium sp.* The findings offer valuable insights for future research into the isolation and development of novel anti-diabetic agents, with potential implications for diabetes management. This work lays a foundation for further exploration into the therapeutic potential of fungal-derived bioactive compounds.

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CONFLICTS OF INTEREST: Nil

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