

Bio-prospecting *Penicilium sp.* isolated from *Aegle marmelos*

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Abstract:

Aegle marmelos (Bael) is a medicinally important plant traditionally valued for its diverse therapeutic applications. Endophytic fungi associated with medicinal plants are recognized as potential sources of biologically active secondary metabolites. The present study was undertaken to investigate the phytochemical composition and multifunctional biological activities of fungal endophytes isolated from different tissues of *A. marmelos*. Preliminary phytochemical screening of *Penicillium sp.* extract revealed the presence of alkaloids, tannins, terpenoids, phenols and flavonoids, along with saponins and steroids. Antimicrobial activity was evaluated by the Kirby–Bauer disc diffusion method against nine bacterial pathogens and *Candida albicans*. The mean MIC of B11 was 23.44 ± 8.2 µg/ml, with the lowest MIC of 15.62 µg/ml observed against several tested pathogens. B11 demonstrated 99.78% antioxidant activity at the highest tested concentration. The α -amylase inhibition assay further demonstrated anti-diabetic potential, with B11 exhibiting an EC₅₀ of 37.38 ± 23.15 µg/ml. The efficient fungal endophytic isolate B11 was morphologically characterized, and the most promising isolate was identified *Penicillium chrysogenum*. The FTIR analysis indicated the presence of hydroxyl, amine, phenolic, alcoholic, aromatic and aliphatic functional groups, while GC–MS analysis identified metabolites including azulene, undecane, citronellol, 1-tridecene and benzo[b]thiophene. Overall, the findings demonstrate that *Penicillium chrysogenum* associated with *Aegle marmelos* possesses significant antimicrobial, antioxidant and α -amylase inhibitory activities. The comparatively low MIC, IC₅₀ and EC₅₀ values of B11 highlight its potential as a promising source of multifunctional bioactive secondary metabolites for further pharmacological and biotechnological investigation.

Keywords: *Aegle marmelos*; endophytic fungi; *Penicillium chrysogenum*; antimicrobial activity; antioxidant activity; α -amylase inhibition.

Introduction:

Aegle marmelos, identified as Bael in India, is a plant of cultural importance in Indian subcontinent. It is popularized by different names such as Bengal quince, golden apple, wooden apple, Japanese bitter orange, stone apple etc. across the globe [1] [2]. Bael has religious significance in India. Also the traces of religious application of bael are found in manuscripts of neighboring countries of South Asian region. As

per the data it is verified that even before 5000 years bael was included in regular diet and medicinal preparations. Historical literature such as Charak Samhita, Ayurveda as well as epic poem Ramayna also mentions the diversified application of bael [3]. Various ayurvedic and folk remedies utilizes therapeutic potential of different parts of *Aegle marmelos* viz, fruit, flower, stem, bark, roots, leaves and seeds. *Aegle marmelos* is a subtropical plant but can also grow in tropical regions till altitude of 1200m. The plant belongs to Rutaceae family. It has gained the importance in the rutaceae family due to its monotypic genus. The fruit of *Aegle marmelos* shows variations in shape and size viz., oval, spherical, pyriform or oblong ranging from 5 to 25 cm in diameter. *Aegle marmelos* is a medium sized, deciduous shrub with height up to 13m long [4]. The fruit of *Aegle marmelos* is proved of great help during Indian summer season due to its moisture content (about 60%) along with its mineral content, fats, fibers, nutritional compositions, fatty acids and amino acids. Along with the fruit, leaf, roots, stem, bark and seeds are also reported to possess medicinally important compounds [5]. However, few studies conducted on different medicinal plants suggest that endophytes colonizing the plant produce therapeutically important secondary metabolites. Endophytes are the micro-organisms inhabiting different parts of plants. Endophytes are known to form symbiotic association with the plant in which exchange of nutrients and secondary metabolites takes place [6]. Endophytes were initially titled for fungi colonizing the plants performing parasitism [7]. Later it was discovered endophytes can be both bacterial and fungal species completing a portion or complete life cycle in mutualistic association with the plant [8]. The present study focus on contribution of endophytes isolated from *Aegle marmelos* in biological properties of host plant.

Method:

Isolation and identification of fungal endophytes:

Diverse Healthy and fresh parts of *Aegle marmelos* viz. stem, leaf, fruit and roots were sampled from different localities in Washim, Maharashtra, India. The collected samples were transferred to microbiology laboratory R.A. College, Washim, Maharashtra, India, for surface sterilization. The sterilized parts were dissected into blocks of 1cm. The dissected samples were inoculated in potato dextrose agar plates. The plates were incubated at room temperature for 5-7 days. After incubation the fungal isolates were pure cultured and identified using lactophenol blue staining [10].

Enrichment of fungal endophytes:

Endophytic fungal isolates were cultured in 100 mL sterilized Potato Dextrose Broth (PDB) in 250 mL conical flasks and incubated at 28°C for 15 days with shaking at 150 rpm. The resulting fungal biomass was filtered, dried, and weighed before homogenization in 10% methanol. An equal volume of ethyl acetate was added, and the mixture was shaken for 10 min and allowed to stand for phase separation. The ethyl acetate fraction containing secondary metabolites was collected using a separating funnel and evaporated to obtain the crude extract. The dried extracts were weighed to determine extract yield, reconstituted in DMSO at 1 mg/ml, and stored at 4°C until further phytochemical and biological analyses [15] [11].

Biological activities of fungal endophytes:

Crude extracts of fungal endophytes were subjected to phytochemical analysis along with different biological activities such as antimicrobial, antioxidant and anti-diabetic.

Antimicrobial activity by Kriby- Bauer disc diffusion method

Antimicrobial activity of crude extracts was performed by disc diffusion method. Sterilized Muller Hinton agar plates were inoculated with fresh cultures of test pathogens using sterile cotton swab. 20µl of fungal crude extracts were then transferred over the sterilized blank filter paper disc. These discs were then transferred to inoculated agar plates under aseptic conditions. Standard antibiotic tetracycline was used as positive control and disc impregnated with methanol was used as negative control. The plates were incubated at 37°C for 24 hrs. After incubation the antimicrobial activity of endophytic fungal crude extracts was measured in terms of zone of inhibition [12]. The antimicrobial activity was categorized as weak, moderate and strong as per the description given by Yang *et al.*, (2019) [9].

Minimum inhibitory concentration

Minimum inhibitory concentration of fungal crude extract was conducted by disc diffusion method using two fold dilution methods. The crude extracts were prepared in series of concentration ranging from 1000 µg/ml to 15.62 µg/ml. 20µl of each concentration was transferred over the sterilized blank filter paper disc. These discs were then placed over Muller Hinton agar plates enriched with test pathogen. The plates were further process for incubation at 37°C for 24 hrs. Effective concentration of fungal crude extract for antimicrobial potential was determined by presence of zone of inhibition [13].

Antioxidant activity by DPPH method

Antioxidant potential of crude extracts was performed using DPPH radical scavenging assay. Methanol extracts of fungal endophytes were prepared by suspending DMSO extract in methanol solvent in 4:1 ratio. For the assay 1 ml of methanol extract of endophytic fungi was mixed with 2ml of methanol DPPH solution following the incubation of 30 min at room temperature in dark. The change of DPPH colour from violet to yellow indicates positive test which is further measured by decrease in absorbance at 517nm in UV Spectrophotometer. Percentage of DPPH radical scavenging activity was calculated using following formula.

$$\% \text{ DPPH scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100$$

A0= Absorbance of the control

A1= Absorbance of fungal extract

Different concentrations of extracts were prepared in 2-fold dilution to calculate the IC₅₀ of fungal crude extracts [14].

Anti-diabetic activity by alpha-amylase inhibition assay

Alpha-amylase activity of fungal crude extract was conducted according to the method described by Jayant and Vijayakumar (2021) [18]. For this assay 40 µl of extract was added in 200 µl of phosphate buffer. 40 µl of alpha amylase enzyme solution was added to the mixture and incubated for 30 min. at 37°C to initiate the enzyme substrate response. Followed by the incubation 50 µl of 1% starch solution is added to the test tubes. The mixture is again incubated at 37°C for 20min. to allow the enzymatic hydrolysis of starch. The reaction was terminated by adding 0.5 ml of DNSA reagent. The test tubes were then heated for 5min. in boiling water bath. 5 ml of distilled water was added to the reaction mixture to make up the volume. Absorbance was measured at 540nm in UV Spectrophotometer. Blank was prepared in similar manner only by replacing fungal crude extract with phosphate buffer. Percent inhibition of fungal crude extract was calculated by following formula:

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

A_{control} = Absorbance of the control reaction

A_{sample} = Absorbance of the reaction containing fungal extract

EC₅₀ concentration of alpha amylase inhibition was determined based on methodology of Olaokun *et al.*, 2013 [19].

FTIR analysis:

Crude extracts of fungal endophytes were subjected to FTIR analysis to identify the functional groups present. The dried crude extracts were combined with potassium bromide (KBr) which is then compressed in small pellet that was used for analysis. As KBr is transparent in infrared light, the sample was irradiated by the light and absorbance at particular frequency was plotted. Absorbance was measured in range of 4000-400 nm for detection of species.

GCMS analysis:

Methanolic purified extracts of the selected endophytic fungal isolates were subjected to GC–MS analysis for the identification of volatile and semi-volatile bioactive metabolites. Helium was used as the carrier gas, and samples were injected at a split ratio of 25:1 with the injector maintained at 270°C. The oven temperature was programmed from 45°C to 150°C, followed by 150–200°C and 200–270°C, with appropriate holding times. The ion source and interface temperatures were maintained at 200°C and 260°C, respectively. Compounds were identified by comparing their mass spectra with reference spectra in the NIST spectral library. The identified metabolites were recorded along with their retention times, molecular weights, molecular formulas and relative peak area percentages for further interpretation.

RESULT:

Isolation and identification of fungal endophytes colonizing different parts of *Aegle marmelos* viz. stem, leaf, fruits and roots were evaluated for the presence of bioactive metabolites having therapeutic

potential. During the identification of all fungal endophytes, it was observed that roots and leaf of *Aegle marmelos* harbors major percentage of population. Molecular identification was performed to identify the most effective strain in addition to morphological characterization. Branched conidiophores with symmetrical arrangement with bottle shaped phialides with globose conidial spores arranged in sequential chains were observed in the microscopy of the efficient strain. The strain was identified as *Penicillium chrysogenum*.

The isolate was enriched in the Potato Dextrose Broth for 2 weeks by agar plug of active culture with frequent shaking in rotatory shaker. During enrichment the colour change of broth was observed from pale yellow to muddy red with growth of fungal mycelial matt. The endophytic fungal mycelial biomass filtered from enriched broth was homogenized to extract the secondary metabolites. The secondary metabolites extracted from fungal mycelia were stored in DMSO for further studies. Dry weight of mycelia and crude extract was calculated in which it was found that isolate B11 identified as *Penicillium chrysogenum*.. weighing 11.6 g. the crude extract isolated form B11 weight approximately 1.7 mg.

Phytochemical analysis of Penicillium chrysogenum Fungal Endophytes

The DMSO extract was converted in to methanolic extract by suspending 4ml of methanol to 1g/ml of extract. The methanolic extract was subjected to phytochemical analysis. It was found that *Penicillium chrysogenum* synthesized alkaloids, tannins, terpenoids, phenols and flavonoid. Presence of saponins, steroids and quinones was also observed in crude extract. The methanolic extract of *Penicillium sp.* was also subjected to antimicrobial, antioxidant and anti-diabetic analysis.

Table 1: Phytochemical analysis of fungal crude extracts

Isolates	Phytochemical tests									
	Alkaloid		Terp	Phe	Flav	Sapo	Ster	Tan	Cou	Quin
	Drag	May								
B11	+	+	+	+	+	+	+	+	-	+
L8	+	+	+	+	+	+	+	+	+	-

Antimicrobial activity by Kirby-Bauer disc diffusion method

Antimicrobial activity of methanolic endophytic fungal crude extract was determined by testing it against 9 bacterial pathogen viz. *Bacillus spp.*, *Enterococcus spp.*, *Proteus spp.*, *Salmonella spp.*, *Klebsiella*, *Pseudomonas*, *E.coli*, and *Shigella*. Antifungal activity of extract was tested against *Candida albicans*. The results showed significance with p-value <0.05. Strong antimicrobial activity was noted for B11 crude extracts which was identified as *Penicillium sp.* Isolate B11 was observed against *Shigella sp.* (21mm). 20mm diameter of zone of inhibition was observed against *Bacillus and Enterococcus sp.* followed by 18 mm zone against *S.aureus* and *Klebsiella sp.* 17 mm zone was recorded against *E.coli*, *Proteus sp.* and

Salmonella sp. Least activity of *Penicillium chrysogenum* was observed against *Pseudomonas* sp. with inhibitory zone of 16 mm. Against *Candida albicans* 17 mm of zone was noted for crude extract. Effective concentration of *Penicillium chrysogenum* crude extract was calculated to be 31.25 µg/ml. against majority of pathogens including *E.coli* and *Pseudomonas* sp. the effective concentration of crude extract was calculated by determining the minimum inhibitory concentration.

Table 2: Antimicrobial activity by Kirby-Bauer disc diffusion method

Fungal Crude Extracts	Zone of inhibition against Pathogens (in mm)										Mean
	<i>E.co.</i>	<i>S.a.</i>	<i>Sal.</i>	<i>Shig.</i>	<i>Pseu.</i>	<i>Pro.</i>	<i>Bac.</i>	<i>Kleb.</i>	<i>Ent.</i>	<i>Cand.</i>	
B11	17	18	17	21	16	17	20	18	20	17	18.1±1.6
L8	18	15	20	21	16	17	19	17	18	15	17.6±2

E.co. = *Escherichia coli*; *S.a.* = *Staphylococcus aureus*; *Sal.* = *Salmonella*; *Shig.* = *Shigella*; *Pro.* = *Proteus*; *Pseu.* = *Pseudomonas*; *Ent.* = *Enterococcus*; *Kleb.* = *Klebsiella*; *Cand.* = *Candida*; *Bac.* = *Bacillus*

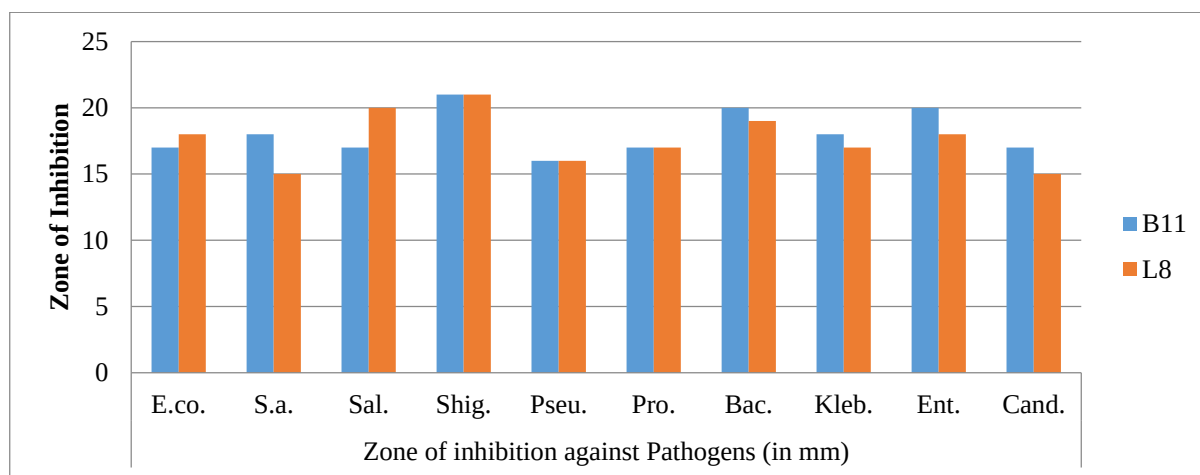
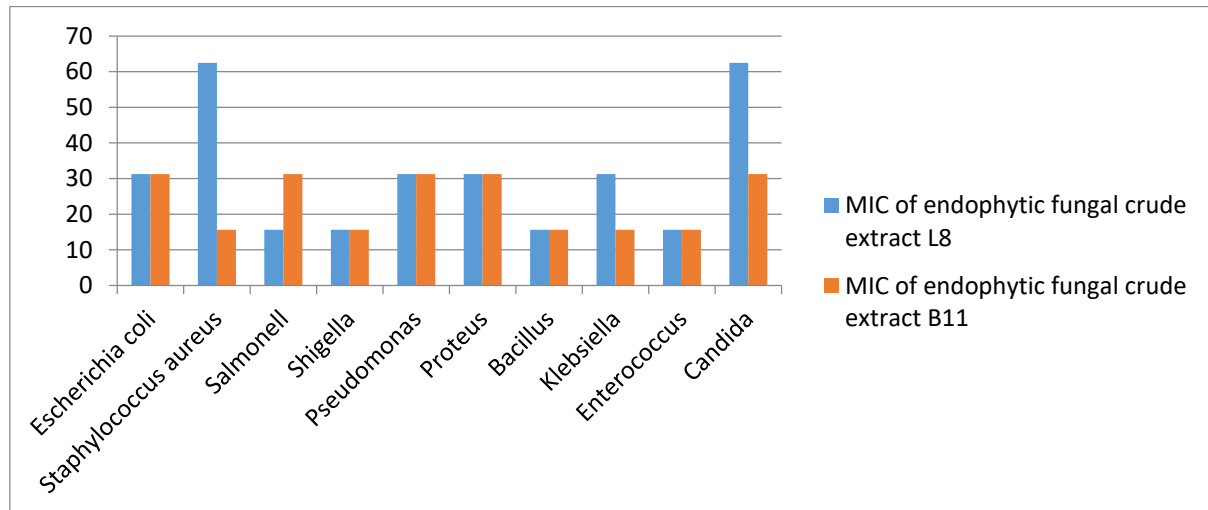


Figure 1: Antimicrobial activity by Kirby-Bauer disc diffusion method

Minimum inhibitory concentrations of *Penicillium chrysogenum* are recorded in following table. A lower MIC value indicates greater antimicrobial potency because a smaller concentration of the extract is required to inhibit visible microbial growth. The results showed significance with p-value < 0.05. The MIC values of the L8 and B11 extracts ranged from 15.62 to 62.5 µg/ml, demonstrating moderate to strong inhibitory activity against the tested pathogens. Among the two extracts, B11 exhibited greater overall antimicrobial potency, as evidenced by its lower mean MIC (23.44 ± 8.2 µg/ml) compared with L8 (31.25 ± 18 µg/ml). The lowest MIC (15.62 µg/ml) was observed for B11 against *Staphylococcus aureus*, *Shigella*, *Klebsiella*, *Bacillus*, and *Enterococcus*. An MIC of 31.25 µg/ml was recorded against *Escherichia coli*, *Salmonella*, *Pseudomonas*, *Proteus*, and *Candida*. The lower mean MIC of B11 (23.44 µg/ml) indicates that the extract is more potent for antimicrobial activity under experimental conditions under the experimental conditions.

Table 3: MIC of *Penicillium* sp. crude extract

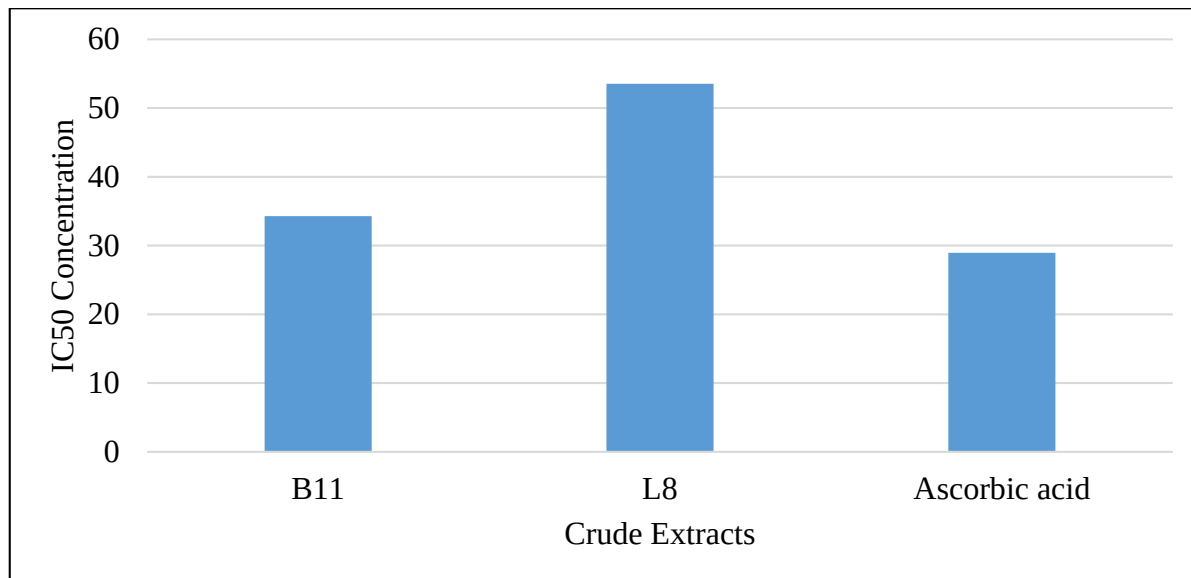
Pathogens	MIC of endophytic fungal crude extract($\mu\text{g/ml}$)	
	L8	B11
<i>Escherichia coli</i>	31.25	31.25
<i>Staphylococcus aureus</i>	62.5	15.62
<i>Salmonell</i>	15.62	31.25
<i>Shigella</i>	15.62	15.62
<i>Pseudomonas</i>	31.25	31.25
<i>Proteus</i>	31.25	31.25
<i>Bacillus</i>	15.62	15.62
<i>Klebsiella</i>	31.25	15.62
<i>Enterococcus</i>	15.62	15.62
<i>Candida</i>	62.5	31.25
Mean	31.248 $\pm$ 18	23.435 $\pm$ 8.2

**Figure 2: MIC of *Penicillium* sp. crude extract****Antioxidant activity by DPPH method**

Antioxidant potential of *Penicillium chrysogenum* isolated from *Aegle marmelos* was analyzed using DPPH radical scavenging activity. In this method positive results are indicated by the reduction of DPPH in the presence of radical scavengers present in the crude extract. The activity can be measured by the decolorizing effect and U.V. spectrophotometry of the sample. Ascorbic acid was used as standard control with IC₅₀ of 28.9 $\mu\text{g/ml}$. The antioxidant activity exhibited by B11 and L8 exhibited significant activity with p-value <0.05. Crude extract B11 exhibited significant antioxidant activity with 99.78% potential and IC₅₀ value of 34.28 $\mu\text{g/ml}$. Similarly, Extract of L8 isolate exhibited IC₅₀ concentration of 53.54 $\mu\text{g/ml}$.

Table 4: Antioxidant activity by DPPH method

Crude extracts	% inhibition for 2 fold concentrations (µg/ml)							IC 50 Concentration (µg/ml)
	15.62	31.25	62.5	125	250	500	1000	
B11	36.99	49.31	56.42	60.37	66.62	79.88	99.78	34.28 ± 7.7
L8	29.39	45.47	51.92	69.21	78.26	83.34	92.32	53.54 ± 18.29
Ascorbic acid	35.9	52.4	60.6	68.8	76.5	88.1	94.2	28.96

**Figure 3: Antioxidant activity by DPPH method**

Anti-diabetic activity by alpha-amylase inhibition assay

α -amylase inhibition assay was performed to determine the anti-diabetic potential of *Penicillium* sp. isolated from *Aegel marmelos*. Positive results of α -amylase inhibition assay were confirmed by measuring absorbance at 540nm using UV-Visible spectrophotometer. Different concentrations of crude extracts were prepared to determine the effective concentration. Acarbose was used as standard for the study with an EC₅₀ value of 39.40 µg/ml. The antioxidant activity exhibited by B11 and L8 exhibited significant activity with p-value <0.05. From the table it is observed that EC₅₀ concentration of B11 crude extract was recorded at 37.38 µg/ml. Crude extract of L8 was recorded as 78.51 µg/ml.

Table 5: Anti-diabetic activity by alpha-amylase inhibition assay

Crude extracts	% inhibition of different Concentration (µg/ml) of fungal crude							EC 50 Concentration (µg/ml)
	15.62	31.25	62.5	125	250	500	1000	
B11	39.35	48.80	54.92	66.46	82.58	93.87	99.39	37.38±23.15
L8	19.56	33.14	43.28	69.51	73.42	89.61	98.88	78.51±29.68
Acarbose	31.89	47.32	57.60	72.59	85.10	91.03	94.79	39.40

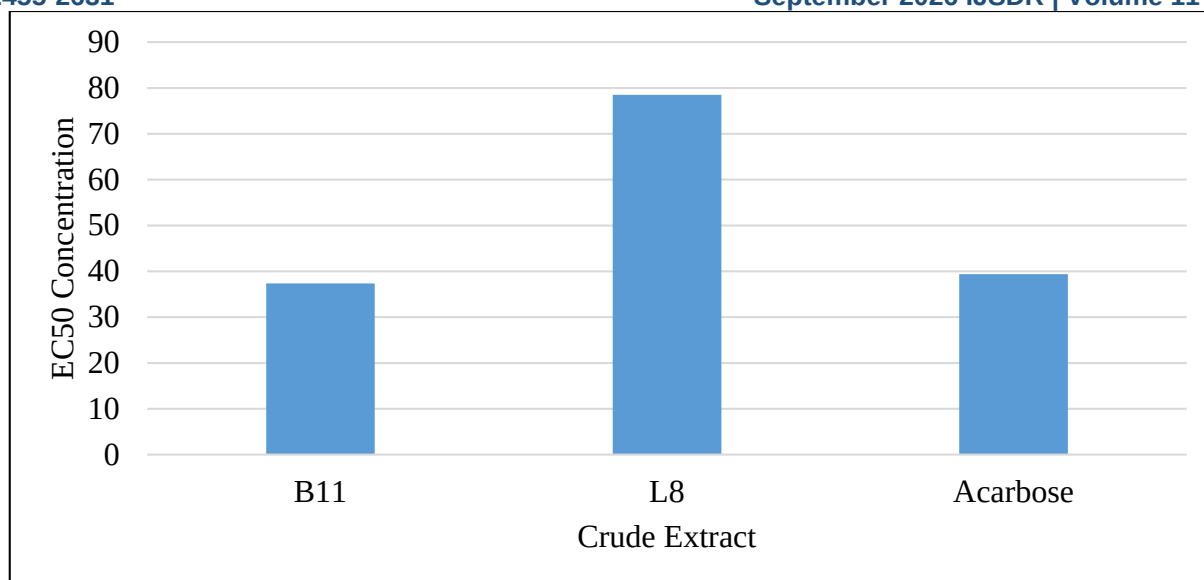


Figure 4: Anti-diabetic activity by alpha-amylase inhibition assay

Further the endophytic fungal crude extract of B11 isolate was purified by TLC chromatographic technique. The purified extracts were subjected to FTIR and GC-MS analysis. FTIR analysis of B11 extracts exhibited broad peak of O-H and N-H at 3537.51 cm^{-1} . Peak at 3391.88 cm^{-1} also indicates similar stretching expressing presence of hydroxyl-containing compounds and proteins. Presence of phenols, alcohols, amines, unsaturated alkenes, aromatic compounds and aliphatic compounds was also recorded in the graph of FTIR. GC-MS characterization of the purified extract detected the presence of Azulene, undecane, Citronellol, 1-Tridecene, Benzo[b]thiophene. Detection of these compounds validates the excellent biological activities expressed by B11 crude extracts.

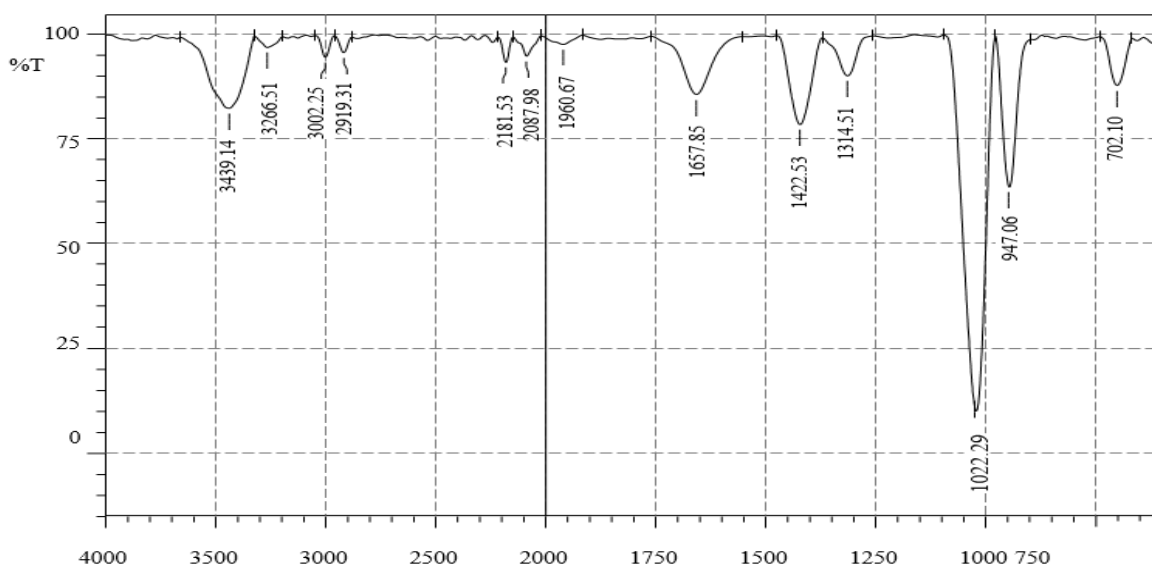


Figure 5: FTIR graph of B11 extract

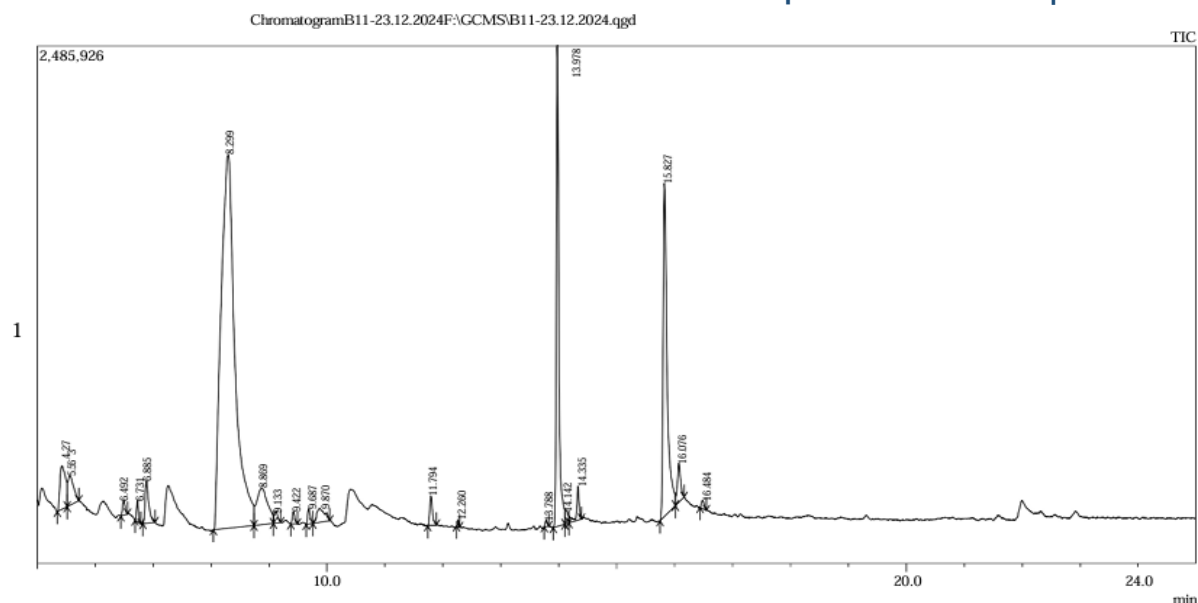


Figure 6: GC-MS of B11 extracts

DISCUSSION:

Endophytes represents an unexplored source of novel secondary metabolites that can be a better alternative to current therapeutic agents. The metabolites produced by fungal endophytes have potent pharmaceutical and industrial importance [16]. In present investigation the endophytes colonizing different tissues of *Aegle marmelos* plant are studied for their biological activities. Form the study it was found that colonizing potential of fungal endophytes was more concentrated in roots and leaf of plants as compared to other tissues like stem and fruit [17]. The tissue dependent variation of colonizing ratio of fungal endophytes may be due to the nutritional requirement, tissue physiology and host-endophyte interaction. Hence, it is possible that fungal endophytes may have colonized roots and leaf tissues of *Aegle marmelos* plant.

Identification of effective isolate B11 as *Penicillium sp.* by morphological and molecular identification is particularly significant and supported by the biological activities exhibited by the isolate. Preliminary phytochemical screening of crude extracts of fungal endophytes detected the presence of alkaloids, phenols, terpenoids, flavonoids and other compounds which was then supported by the FTIR analysis. Simultaneous presence of phytochemicals represents that biological activities of fungal crude extract are results of more than one compound working together. Presence of phenols and flavonoids supports the positive results gained during antioxidant activity. While presence of terpenoids and alkaloids reports the antimicrobial and anti-diabetic activity. According the findings of antimicrobial activity, diversity in readings could be due to variation in cell wall structure. The ability of B11 extract to inhibit both gram positive and gram negative test pathogens along with MIC assay provides the antimicrobial potential of fungal crude extracts at low concentrations.

CONCLUSION:

Penicillium sp. isolated as endophytic fungi from *Aegle marmelos* plant has multifunctional therapeutic profile. Among the tested extracts, B11 consistently exhibited superior activity, as reflected by its lower mean MIC (23.44 $\mu\text{g/mL}$), lower antioxidant ($\text{IC}_{50} = 34.28 \mu\text{g/mL}$), and lower α -amylase ($\text{EC}_{50} = 37.38$

µg/mL). The antioxidant IC₅₀ of B11 was also relatively close to that of ascorbic acid, while its α -amylase EC₅₀ was comparable to that of acarbose. The correlation between the biological activities and the phytochemical, FTIR and GC–MS findings suggests that the observed activity may be associated with a complex mixture of secondary metabolites produced by the endophytic fungus. The ability of a single endophytic isolate to exhibit antimicrobial, antioxidant and enzyme-inhibitory activities highlights the biotechnological significance of *Penicillium* sp. associated with *A. marmelos*.

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REFERENCE:

- [1] Misra KK (1999). "Bael". NewCROP, the New Crop Resource Online Program, Department of Horticulture and Landscape Architecture, Center for New Crops & Plant Products, Purdue University, W. Lafayette, IN. Retrieved 20 January 2016.
- [2] Porcher Michel H. *et al.* 1995 - 2020, Sorting Aegle Names. Multilingual Multiscript Plant Name Database (M.M.P.N.D) - A Work in Progress. School of Agriculture and Food Systems. Faculty of Land & Food Resources. The University of Melbourne. Australia.
- [3] Pathirana, C. K., Madhujith, T., & Eeswara, J. (2020). Bael (*Aegle marmelos* L. Corrêa), a medicinal tree with immense economic potentials. *Advances in Agriculture*, 2020(1), 8814018.
- [4] Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Anthony, S. (2009). Agroforestry database. *a tree reference and selection guide version, 4*.
- [5] Sharma, N., Radha, Kumar, M., Zhang, B., Kumari, N., Singh, D., Chandran, D., Sarkar, T., Dhumal, S., Sheri, V., Dey, A., Rajalingam, S., Viswanathan, S., Mohankumar, P., Vishvanathan, M., Sathyaseelan, S. K., & Lorenzo, J. M. (2022). *Aegle marmelos* (L.) Correa: An Underutilized Fruit with High Nutraceutical Values: A Review. *International journal of molecular sciences*, 23(18), 10889. <https://doi.org/10.3390/ijms231810889>
- [6] Gouda, S., Das, G., Sen, S. K., Shin, H. S., & Patra, J. K. (2016). Endophytes: a treasure house of bioactive compounds of medicinal importance. *Frontiers in microbiology*, 7, 1538.
- [7] Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., ... & Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and molecular biology reviews*, 79(3), 293-320.
- [8] Singh, R., & Dubey, A. K. (2015). Endophytic actinomycetes as emerging source for therapeutic compounds. *Indo Global J Pharm Sci*, 5(2), 106-116.
- [9] Yang X, Wang D, Zhou Q, Nie F, Du H, Pang X, Fan Y, Bai T, Xu Y. Antimicrobial susceptibility testing of Enterobacteriaceae: determination of disk content and Kirby-Bauer breakpoint for ceftazidime/avibactam. *BMC Microbiol*. 2019 Nov 1;19(1):240. doi: 10.1186/s12866-019-1613-5. PMID: 31675928; PMCID: PMC6824082.
- [10] Barnett, H. L., & Hunter, B. B. (1998). Illustrated genera of imperfect fungi. The American phytopathological society. *US Department of Agriculture, Agricultural Research Service, Washington State University, Pullman. APS Press. USA. St. Paul, Minnesota USA. 218p*.
- [11] Sharma, D., Pramanik, A., & Agrawal, P. K. (2016). Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D. Don. *3 Biotech*, 6(2), 210.
- [12] Pavithra, N., Sathish, L., & Ananda, K. (2012). Antimicrobial and enzyme activity of endophytic fungi isolated from *Tulsi*. *Journal of Pharmaceutical and Biomedical Sciences (JPBMS)*, 16(16), 2014.

- [13]Hulankova, R. (2024). Methods for Determination of Antimicrobial Activity of Essential Oils In Vitro—A Review. *Plants*, 13(19), 2784. <https://doi.org/10.3390/plants13192784>
- [14]Gurgel, R. S., de Melo Pereira, D. Í., Garcia, A. V. F., Fernandes de Souza, A. T., Mendes da Silva, T., de Andrade, C. P., Lima da Silva, W., Nunez, C. V., Fantin, C., de Lima Procópio, R. E., & Albuquerque, P. M. (2023). Antimicrobial and Antioxidant Activities of Endophytic Fungi Associated with *Arrabidaea chica* (Bignoniaceae). *Journal of fungi (Basel, Switzerland)*, 9(8), 864. <https://doi.org/10.3390/jof9080864>
- [15]Sadrahi, N., Daoud, H., Zerroug, A., Dahamna, S., & Bouharati, S. (2013). Screening of antimicrobial and antioxidant secondary metabolites from endophytic fungi isolated from wheat (*Triticum durum*). *Journal of plant protection research*, 53(2).
- [16]Debbab, A., Aly, A. H., & Proksch, P. (2013). Mangrove derived fungal endophytes—a chemical and biological perception. *Fungal diversity*, 61(1), 1-27.
- [17]Arnold, A. E. In *Endophytic Fungi: Hidden Components of Tropical Community Ecology*; Schnitzer, S.; Carson, W., Ed.; Tropical Forest Community Ecology; Blackwell Scientific, Inc: Malden, MA, 2008.
- [18]Jayant, K. K., & Vijayakumar, B. S. (2021). In-Vitro anti-oxidant and anti-diabetic potential of endophytic fungi associated with *Ficus religiosa*. *Italian Journal of Mycology*, 50, 10-20.
- [19]Olaokun, O. O., McGaw, L. J., Eloff, J. N., & Naidoo, V. (2013). Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity and polyphenolic content of extracts of ten African *Ficus* species (Moraceae) used traditionally to treat diabetes. *BMC Complementary and Alternative medicine*, 13(1), 94.