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Exploring the Endophytic and Phytochemical Landscape of *Aegle marmelos*: From Ethnomedicine to Biotechnological Innovation

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Abstract

Aegle marmelos is a well-known medicinal plant in Ayurveda with a variety of pharmacological qualities due to its rich phytochemical profile. This article discusses the phytoconstituents and endophytes found in leaves, bark, roots, fruits, and seeds of different plants. It also emphasises how endophytes, especially fungi and actinomycetes, are linked to A. marmelos and how they can mimic and enhance its therapeutic potential. Notable antimicrobial, antioxidant, anticancer, antidiabetic, antiviral, and enzyme-inhibitory properties have been observed in endophytic species such as Fusarium, Penicillium, Aspergillus, Trichoderma, and Xylaria. There are new opportunities for sustainable drug discovery and therapeutic development due to the growing evidence of horizontal gene transfer and phytochemical convergence between endophytes and their hosts.

Keywords: *Aegle marmelos*, Bael tree, Rutaceae, endophytes.

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Introduction

Aegle marmelos, commonly known as golden apple or bael, is native to Southeast Asia and India and belongs to the family Rutaceae, which comprises approximately 120 genera and 1,000 species. *Aegle marmelos* is a medium-sized deciduous tree that typically reaches a height of 12–15 meters. It exhibits slow but distinct growth, with a short trunk, soft and peeling bark, and drooping lower branches. The leaves are petiolate, aromatic, and trifoliate, arranged alternately. They are exstipulate and palmately compound. The inflorescences are terminal and axillary scorpioid cymes. The flowers are aromatic, bisexual, actinomorphic, pentamerous, and hypogynous. They lack bracteoles (ebracteolate) and have a pedicel. The calyx is synsepalous, five-lobed, and deciduous. The corolla is apopetalous, consisting of five greenish-yellow petals that are significantly longer than the calyx. The androecium is polyandrous, with numerous stamens that have short filaments and dithecous, basifixed anthers that dehisce longitudinally and are introse. The ovary is ovoid, five-lobed, and syncarpous with five locules. It features axile placentation, a short style, and a capitate stigma (KyawSoe *et al*., 2004). Various parts of the tree are rich in bioactive compounds, including alkaloids, cardiac glycosides, coumarins, terpenoids, saponins, tannins, flavonoids, steroids, eugenol, lupeol, cineole, citronellal, cuminaldehyde, marmesin, auraptene, skimmianine, citral, luvangentin, anhydromarmelin, aegeline, marmesinine, marmelosin, marmelin, marmelide, psoralen, scopoletin, fagarine, limonene, betulinic acid, imperatorin, and cineole (Rathee *et al*., 2018; Raheja *et al*., 2019; Seemaisamy *et al*., 2019). Endophytic microbes may be able to create compounds that mimic the characteristics of A. marmelos. Over a lengthy period of co-evolution with host plants, endophytes have developed a perfectly compatible symbiotic relationship by adapting to niches and using gene control (Kumari *et al*., 2018). These microbes have the ability to increase their hosts' resistance to biotic (insects, pathogens, herbivores, etc.) and abiotic (drought, flood, high salt, improper temperature, etc.) stresses (Meshram *et al*., 2013; Kumari *et al*., 2018; Panigrahi *et al*., 2018). Additionally, endophytes have the ability to secrete certain bioactive substances that effectively reduce the prevalence of autoimmune diseases, diabetes, arthritis, malaria, and tuberculosis (Shah *et al*., 2016; Abdel *et al*., 2016; Rajamanikyam *et al*., 2017; Ateba *et al*., 2018; Saini *et al*., 2018). Despite their enormous biological potential, endophytic microbes from numerous Indian medicinal plants have not yet been identified, which has led to additional research in this field (Patil *et al*., 2015). It is believed that only a small number of microorganisms can grow and survive in medicinal and aromatic plants due to their chemical compositions (Gawas *et al*., 2010).

Origin and Distribution of *Aegle marmelos*

The Bael tree originated in Central India and the Eastern Ghats. It is native to the Indian subcontinent and is primarily found in tropical and subtropical areas. Up to 500 meters above sea level, the tree can also be found growing wild in the lower Himalayan ranges. Bael grows along the Deccan Plateau, the East Coast, the Himalayan foothills, Uttar Pradesh, Bihar, Chattisgarh, Uttaranchal, Jharkhand, and Madhya Pradesh [Singh *et al*., 2000; Purohit *et al*., 2004). This tree and other trees in the area were observed by Hiuen Tsiang, a Chinese Buddhist pilgrim who travelled to India in 1629 A.D. Sambamurthy *et al*., 1989). Additionally, some Egyptian gardens in Trinidad and Surinam grow it. Bael specimens have been acquired and are kept at the Citrus Collection In Florida (Jauhari *et al*., 1969). In Burma, bael fruit has long been used to make paint (Parmar *et al*., 1982). The tree has been used for its hypoglycemic properties in Sri Lanka and for fertility control and antiproliferative purposes in Bangladesh (Karunanayake *et al*., 1984; Lampronti *et al*., 2003; Kala *et al*., 2006). In 1959, bael fruit was brought to Europe (Knight *et al*., 1980). According to reports, the tree is also grown in Java, Ceylon, Northern Malaya, and the Philippine Island, where it produced its first fruit in 1914 (Morton *et al*., 1987).

***Aegle marmelos* in Ayurveda**

Since 5000 B.C., people have utilised fruits as food and medicine (Baliga *et al*., 2011). Ayurveda and other traditional medical systems have made extensive use of these fruits and their parts (Axay *et al*., 2012). The plant's leaf is used to treat asthma and jaundice (Bhar *et al*., 2019). They are also effective in treating leucorrhea, constipation, deafness, and conjunctivitis. Additionally, bowel syndrome is treated with leaf powder (Atul *et al*., 2012). In a similar vein, unripe fruit is used to cure abscesses, while fruit pulp helps with intestinal, urinogenital, and other issues related to indigestion (Kumar *et al*., 2012). In India, burn injuries are treated with a concoction of mustard oil and powdered fruit (Jyotsana *et al*., 2010). The plant's flower is used to treat epilepsy and wound healing because of its antiseptic and astringent qualities (Gautam *et al*., 2014). The tree's root and bark can also help with melancholy, heart palpitations, and intermittent fever. One of the most crucial ingredients in the creation of the well-known Ayurvedic medication "Dashmula," which has several advantages, including healthy nervous system operation, is bale tree root (Jyotsana *et al*., 2010).

In the Ayurvedic medical system, *Aegle marmelos* has been used extensively. The majority of Bael's components, including the root, stem, leaf, fruit, and seed, are thoroughly described. The chemical classes, structures, and bioactivities of epicarp phytoconstituents were determined through fractionation, isolation, and characterisation during a study. The fruit-shell's methanolic extract contained therapeutically important classes of compounds, including polyphenols, glycosides, sterols, and carbohydrates. The similarity index (SI) served as the basis for the phytoconstituents' likely identities. GC-MS spectra revealed 209 compounds in total, of which 59 could be identified by comparison with library compounds. This finding implied that there was a good chance of discovering new compounds from this source. Six compounds were reported from bael: 4-hydroxybenzeneacetic acid, 5-oxo-pyrrolidine-2-carboxylic acid methyl ester, trans-sinapyl alcohol, DL-proline, 5-oxo-methyl ester, 5-(hydroxymethyl)-2-furaldehyde, and 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one. Better activities for preventing lipid peroxidation and shielding cells from oxidative stress were shown by compounds recovered in butanol and aqueous fractions. Five pure compounds and sixteen partially purified compounds were identified. Several spectral techniques were used to structurally characterise the pure compounds. Quercetin, benzoic acid, and

1,2-dihydroxybenzene were found to be three of the five isolated compounds. The compounds had shown better anti-oxidant activity than the standard molecules, Ascorbic acid and Tocopherol. Strong anti-inflammatory, anti-diabetic, and anti-aging properties were demonstrated by the purified compound, AMM4, outperforming the reference compounds. AMM5 also demonstrated strong anti-aging properties (Dubey *et al*., 2022).

Phytoconstituents found in *Aegle marmelos*

Scientists have long recognised the role that plants and the compounds they generate play in curing illness. Plants continue to be the primary ingredient in more than 25% of all prescription medications (Qadry *et al*., 2004). According to reports, bael contains a variety of coumarins, alkaloids, steroids, and essential oils. Coumarins including scoparone, scopoletin, umbellliferone, marmesin, and skimming are found in roots and fruits. Additionally, fruits contain alkaloids like aegeline and marmelline, as well as xanthotoxol, imperatorin, and alloimperatorin. It also contains polysaccharides that may be obtained through hydrolysis, such as galactose, arabinose, uronic acid, and L-rahaminose. *Aegle marmelos* has been found to contain a variety of carotenoids, which give fruit its pale yellow hue. The main medicinally active components of the bael plant are umbelliferone, marmelosin, and skimmianine. Other minor components include carotenoids, sitosterol, ascorbic acid, tannins, crude fibres, α-amyrin, and crude proteins. In addition to these chemical constituents, over 100 compounds have been isolated; these include aegelin, lupeol, cineole, citral, citronellal, cuminaldehyde, eugenol, marmesinin, marmelosine, luvangetin, aurapten, psoralen, marmelide, fagarine, marmin, and tennins. It has been demonstrated that these compounds are biologically active against a variety of major and minor diseases (Sharma *et al*., 2007; Maity *et al*., 2009; Lambole *et al*., 2010; Dhankhar *et al*., 2011; Sharma Ganesh N *et al*., 2011). Table 1 summarises the list of chemical components found in the various sections of *Aegle marmelos*.

Table 1: Chemical components found in the various sections of *Aegle marmelos* (Riyanto *et al*, 2001; Qadry *et al*., 2004; Phuwapraisirisan *et al*., 2008; Suvimol *et al*., 2008; Yadav *et al*., 2009; Johnsonet *et al*., 2010; Nugroho Agung *et al*., 2010; Laphookhieo *et al*., 2011; Nugroho *et al*., 2011; Nugroho Agung *et al*., 2011; Sharma Ganesh N *et al*., 2011).

Part of Plant	Chemical Constituents
Bark	Fagarine Furoquinoline Marmin Alkaloids
Leaf	Glycoside O-isopentenyl Citronellal Skimmianine Hallordiol Mameline Euginol Marmesinin Aegelin Lupeol Cineol Citral Aeglin Rutin γ-sitosterole β-sitosterol Flavone Cuuminaldehyde phenylethyle cinnamamides Glycoside
Fruit	Psoralen Luvangetin Aurapten Marmelide Marmelosin Tannin Phenol
Seed	A-D-phellandrene Essential oil – D- limonene Cineol Citronellal P-cyrnene Citral Cumin aldehyde
Root	Coumarins Halopine Alkaloid Terpines

Antiviral Potential of *Aegle marmelos*

Viral infections have grown to be a major problem in recent years, leading to unforeseen health issues all over the world. A wealth of nutrients and medications for the prevention and treatment of different viral diseases can be found in medicinal plants and the phytochemicals they contain. According to a study that used in silico molecular docking, selvin, a bioactive compound present in the Bael plant, has the ability to inhibit several SARS-CoV-2 targets. The substance demonstrated the ability to inhibit the receptors for the SARS-CoV-2S protein, COVID-19 main protease, and the free SARS-CoV-2 main protease enzyme (Nivetha R. *et al*., 2021). Similar to this, various bioactive compounds from the Bael fruit were assessed for their antiviral activity against human coxsackieviruses B1–B6 in the study conducted by Badam *et al*. The plaque inhibition assay was used to determine the inhibitory concentrations of these compounds, and the results showed that marmelide was the most effective viricidal agent. Marmelide disrupted the initial stages of the virus's replication cycle, but these concentrations showed no toxicity to host cells. According to the study, bael compounds—in particular, marmelide—have the potential to act as antiviral agents against coxsackieviruses (Badam *et al*., 2002; Chhetri *et al*., 2021). The potential of *Aegle marmelos* (Bael) extracts from its leaves and fruits for their total phenolic and flavonoid contents, antioxidants, and antibiofilm activity, as well as in ovo antiviral activity against Newcastle disease virus (NDV), was also examined in the study conducted by Andleeb *et al*. The findings demonstrated the extracts' strong antioxidant properties and high concentrations of TPC and TFC. Molecular docking studies revealed a good interaction with the HN protein, and the extracts also demonstrated promising antiviral activity against NDV. According to these results, A. marmelos may be a viable treatment for NDV (Andleeb *et al*., 2021).

4. Various types of Endophytes found in *Aegle marmelos*

In a recent study on the endophytic fungal community associated with *Aegle marmelos*, a total of 20 distinct endophytic fungal isolates were recovered from different plant parts, including roots, stems, and leaves (Rajeshwari *et al*., 2024). The characterization of these isolates was carried out based on colony morphology, growth rate, and conidial septation, which allowed for a preliminary identification of the fungal taxa (Table 2). A small percentage of the identified fungal endophytic cultures are Mucoromycota, whereas the majority are Ascomycota. However, the Ascomycota fungi are divided into six orders. Root samples had the most fungal endophytes, followed by stem samples. Some of these have been identified as Penicillium, Trichoderma, Monolinia, Cladosporium, Alternaria, and Rhizopus, while the majority have been identified as Fusarium and Aspergillus. The Fusarium sp. displayed a cloudy, cottony, white colony. In contrast, the isolates' back view revealed a creamy white to orange colour. Under a microscope, oval-shaped conidia with conidial septations and pointed ends were visible. Aspergillus sp. isolates displayed a range of colony morphologies. Nonetheless, the colony's texture remained consistent, ranging from velvety to woolly. Colonies were also creamy white in the rear view. The isolates were globose-shaped and septation-free upon microscopic inspection. Conidia were growing quickly, and in certain instances, as the incubation period increased, their colour darkened. A small number of Trichoderma isolates were also found, some of which displayed a colony with a greenish woolly texture and a white periphery. A creamy white colony was seen from the rear. It showed branched structure on performing microscopy. The greenish-grey, velvety-textured, leaf-shaped conidia of some Alternaria were visible from its stop. When the penicillium first began to grow, it had a white centre, a green colour, and a velvety texture. After the incubation period was over, it changed to a brown colour, a woolly texture, and a microscopic conidial structure. Fusarium fungal species were found to be the most prevalent among the 79 endophytic isolates from *Aegle marmelos*. (Gond *et al*., 2007). All of the isolated fungal species are members of the Ascomycota, they added. Along with isolates of Curvularia species, it was also reported that the endophytic fungal population of *Aegle marmelos* contained isolates of Alternaria. Additionally, they noted that these endophytes exhibited strong antioxidant properties (Mani *et al*., 2015).

Table 2. Endophytic fungal isolates from different plant parts, including roots, stems, and leaves of *Aegle marmelos* (Rajeshwari *et al*., 2024)

Sr. No	Isolate	Growth	Conidial Morphology	Front View	Back View	Possible Species
1	FI1	Fast	Circular conidia, macroscopic unbranched structure	Green/grey colony, woolly texture	Creamy white	*Aspergillus*
2	FI2	Moderate	Leaf shape conidia, macroscopic structure	Greenish grey colour, velvety texture	Blackish grey	*Alternaria*

Sr. No	Isolate	Growth	Conidial Morphology	Front View	Back View	Possible Species
3	FI3	Fast	Umbrella shaped conidia, macroscopic structure	Cottony white, black spores	Creamy white	*Rhizopus*
4	FI4	Fast	Sickle shaped, septated conidia, microscopic structure	White colony, cottony texture	Orange	*Fusarium*
5	FI5	Moderate	Tube like conidia with septations, macroscopic structure	Brown centre, white periphery, cottony texture	Creamy white	*Alternaria*
6	LI1	Fast	Shield shape conidia	Greyish, velvety texture	Black	*Cladosporium*
7	LI2	Moderate	Oval shaped, septated conidia	White, cottony texture	Creamy white	*Fusarium*
8	LI3	Fast	Oval shape, septated conidia	Greenish centre, white periphery, velvety texture	Yellowish white	*Fusarium*
9	LI4	Moderate	Oval shape, septated conidia, microscopic spores	Greyish white, velvety texture, cloud shape	Creamy white	*Fusarium*
10	LI5	Fast	Microscopic conidia, oval shaped, septated	Reddish white in colour with cottony texture	Dark red	*Fusarium*
11	RI1	Slow	Shield shaped conidia, highly dispersed	Blackish grey, white periphery, velvety	Blackish grey	*Cladosporium*
12	RI2	Fast	Oval shaped, septated conidia	Brownish centre, white periphery, cottony	Orange	*Fusarium*
13	RI3	Fast	Dense conidia, branched conidiophores	Green colour, white periphery, cottony white	Yellowish white	*Trichoderma*
14	RI4	Moderate	Leaf shape conidia, microscopic	Cloudy white, velvety texture	Creamy white	*Alternaria*
15	RI5	Fast	Unbranched conidia, globose, non-septate	Black colour, woolly texture	Creamy white	*Aspergillus*
16	SI1	Fast	Oval shape, septated conidia	Cottony white	Orange/yellowish	*Fusarium*
17	SI2	Moderate	Circular unseptated conidia	Greenish grey, cottony colony	Blackish centre, white periphery	–
18	SI3	Moderate	Flower shaped conidia, microscopic spores	Greyish blue colony, white corner, velvety	Yellowish white	*Penicillium*
19	SI4	Fast	Oval shaped, septated conidia, microscopic spores	White colour, cottony texture	Yellowish white	*Fusarium*
20	SI5	Fast	Leaf shape conidia, microscopic, without septations	Greenish white, woolly texture, waves present	Creamy white	*Trichoderma*

Gond *et al*. (2007) and Mani *et al*. (2015) previously reported that all isolated endophytic fungi from A. marmelos belonged to the Ascomycota, including species of Fusarium, Alternaria, and Curvularia. Importantly, these endophytes demonstrated notable antioxidant potential, indicating their possible application in bioprospecting for natural antioxidants and bioactive compounds.

Free Radical Scavenging Property of Endophytic Actinomycetes in Bael

The ability of an endophytic actinomycete derived from *Aegle marmelos* to inhibit enzymes in vitro was examined. To create different concentrations (100–1000µg/ml), the supernatant was extracted in ethyl acetate. Alpha-amylase and alpha-glucosidase were found to be 50% inhibited (IC50) by the extract at 1950.71 ± 0.11µg/ml and 391.38 ± 0.09µg/ml, respectively. The reducing activity and free radical scavenging (hydroxyl radical, superoxide anion, and nitric oxide free radical) capabilities of ethyl acetate extracts were also evaluated. The extract's total phenol contents were found to be 6.47±0.95 mg/g (gallic acid equivalents) and 42.11±1.88 mg/g (catechol equivalents). The study revealed that the postprandial blood glucose levels could be controlled using endophytic actinomycetes from *Aegle marmelos* without the need for synthetic α-amylase and α-glucosidase inhibitors. Like its host plant, the endophyte can fight off oxidative stress and enzyme activity. As the concentration of the ethyl acetate extract increased, a lovely upward trend was seen in the α-amylase inhibition results. However, a downward trend was noted when extract was used to inhibit α-glucosidase. After purification, it could be a useful treatment for enzymes that break down starch. The pharmaceutical industry, which depends on readily available inoculums for its operations, can also benefit from the use of microbes as reservoirs of bioactive substances (Saini *et al*., 2022).

Antioxidant Capacity of Endophytes from *Aegle marmelos*

A comprehensive investigation was conducted to evaluate the endophytic fungal diversity and antioxidant potential from two medicinal plants, Ocimum sanctum (holy basil) and *Aegle marmelos* (bael). In this study, researchers examined a total of 200 leaf segments from O. sanctum and 720 tissue segments (including bark, leaves, and stems) from A. marmelos to isolate and characterize their endophytic fungal communities. As a result, 147 fungal endophytes were isolated from O. sanctum, while A. marmelos yielded a more substantial number, with 569 fungal isolates. The data revealed that tissue type and plant part significantly influenced the colonization frequency and diversity of endophytes, although the effect of tissue origin was comparatively less pronounced than expected.

To evaluate the antioxidant potential of these isolates, a multi-assay in vitro screening approach was employed. The antioxidant activity of selected fungal extracts was tested through various biochemical assays, indicating significant variability among isolates in their free radical scavenging capabilities. Subsequently, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on the ethyl acetate extracts of selected endophytes to identify the bioactive compounds. The analysis revealed the presence of important secondary metabolites, including phenolic compounds and diketopiperazines a class of cyclic dipeptides known for their broad pharmacological potential.

Further, the biological relevance of these identified compounds was explored using AutoDock Vina, molecular docking software. Through in silico docking studies, diketopiperazines were predicted to effectively bind to the active site of the human heat shock protein 90 (Hsp90), a molecular chaperone associated with cancer progression. These results suggest that some endophytic metabolites, particularly from Penicillium spp., could act as potential inhibitors of Hsp90, indicating their possible application in anticancer therapy. The study also incorporated the Plackett–Burman design, a statistical tool used to determine the critical media components influencing the antioxidant activity of fungal isolates. This approach enabled the researchers to optimize the fermentation parameters by identifying the most influential growth conditions that enhance bioactive metabolite production.

To verify the morphological identification and phylogenetic placement of selected fungal isolates, the Internal Transcribed Spacer (ITS) region of the ribosomal DNA (rDNA) was amplified and sequenced. Molecular identification revealed that Mycelia sterilia (GenBank Accession No. KC560013) from O. sanctum and Penicillium sp. (KC560012) from A. marmelos were the most potent antioxidant-producing endophytes among all isolates screened. This integrated approach combining morphological, molecular, biochemical, and computational techniques highlighted the biotechnological potential of endophytic fungi from medicinal plants. Specifically, it demonstrated that endophytes not only contribute to plant health and stress resistance but also produce metabolites with promising pharmacological applications, including anticancer activity.

Antidiabetic Potential of Endophytes from Momordica charantia and *Aegle marmelos*

A study examined the potential antidiabetic effects of endophytic fungi that were isolated from Momordica charantia and *Aegle marmelos*. The results showed that these fungi generate secondary metabolites that can block α-amylase, a crucial enzyme for the metabolism of carbohydrates. Fusarium oxysporum showed the highest inhibitory activity (53.8%) among the four isolates, indicating that its metabolites are the most effective at controlling postprandial glucose levels. This is consistent with previous research showing that endophytic fungi have the bioactive capacity to produce enzyme inhibitors. Although less effective than Fusarium oxysporum, the moderate inhibitory activities seen in Aspergillus niger (36.5%) and Aspergillus versicolour (30.7%) imply that these fungi also produce metabolites with antidiabetic qualities. However, Penicillium verhagenii's lower activity (19.2%) suggests that endophytic fungi differ in their metabolic profiles and production of bioactive compounds. Through their α-amylase inhibitory activity, this study shows that endophytic fungi isolated from Momordica charantia and *Aegle marmelos* have significant antidiabetic potential. Among the isolates, Fusarium oxysporum showed the highest level of enzyme inhibition, making it the most promising candidate. These results demonstrate the potential of endophytic fungi as a long-term supply of organic bioactive substances for the treatment of diabetes (Athharv *et al*., 2025).

Bioactive Metabolite and Biological Activities of Endophytic Aspergillus flavus L7 Isolated from *Aegle marmelos*

A recent investigation focused on isolating and characterizing an endophytic fungal strain, Aspergillus flavus L7, from the leaf tissues of *Aegle marmelos*. To ensure the endophytic origin of the isolate, only healthy, symptom-free leaves were selected. These samples underwent a rigorous surface sterilization protocol to eliminate epiphytic microorganisms and confirm the internal (endophytic) source of the fungus. Upon cultivation, the isolate produced brown to yellow pigmented colonies. Microscopic examination revealed globose to subglobose conidia ranging from 3 to 4.5 µm in diameter. Taxonomic identification was based on conidiophore structure, hyphal morphology, and colony characteristics, which collectively confirmed the strain's identity as Aspergillus flavus.

To assess its bioactivity, the partially purified ethyl acetate extract of A. flavus L7 was screened against a panel of human pathogenic bacteria and fungi. The extract exhibited notable antimicrobial activity, with zones of inhibition measuring 10–18 mm against bacteria and 15–23 mm against fungi. Among bacterial strains, Staphylococcus aureus exhibited the greatest sensitivity to the extract, whereas Candida albicans showed the largest zone of inhibition (23 mm) among fungal pathogens. Although the antifungal agent fluconazole was more effective against Aspergillus niger, the A. flavus extract demonstrated greater efficacy than some standard antibiotics in combating bacterial pathogens. Beyond its antimicrobial properties, the extract was also tested for its antioxidant capacity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The results showed that at a concentration of 700 µg/mL, the extract achieved a maximum scavenging activity of 64.53%, indicating a dose-dependent antioxidant effect. This suggests the presence of bioactive compounds capable of donating hydrogen atoms to neutralize free radicals, thereby contributing to oxidative stress reduction. The study emphasizes that Aspergillus flavus L7, an endophyte from *Aegle marmelos,* possesses both strong antimicrobial and antioxidant potential. Notably, this may be the first report of such activities being associated with an endophytic A. flavus isolated from this medicinal plant (Patil *et al*., 2015). These findings have broader implications. Endophytic fungi like A. flavus L7 could serve as sustainable and alternative sources of natural antioxidants, helping to reduce dependency on overexploited or slow-growing medicinal plants, thus supporting biodiversity conservation. Additionally, microbial production of bioactive metabolites offers a more practical and cost-effective approach to accessing these compounds, enhancing their commercial viability and accessibility (Strobel *et al*., 2004). Interestingly, it has been hypothesized that some endophytes acquire the ability to produce plant-like secondary metabolites through horizontal gene transfer from their host plants, which could explain the similarity in bioactivity (Wu *et al*., 2001; Strobel *et al*., 2004).

Antifungal Endophytic Metabolites

A study successfully isolated a novel anthraquinone derivative, identified as 1-methyl-2-(3′-methyl-but-2′-enyloxy)-anthraquinone, from the seeds of *Aegle marmelos* Correa, a traditional medicinal plant. The compound's structure was elucidated using an array of advanced spectroscopic techniques, including UV, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, and mass spectrometry. These methods confirmed its structural uniqueness and purity (Mishra *et al*., 2010). The isolated compound underwent in vitro screening against several pathogenic fungi, particularly species of Aspergillus and Candida albicans. The biological assays used included:

Disc Diffusion Assay (DDA): MIC = 6.25 µg/disc

Microbroth Dilution Assay (MDA): MIC = 31.25–62.5 µg/ml

Spore Germination Inhibition Assay: MIC = 31.25 µg/ml

Though amphotericin B (MIC = 2.4 µg/disc) was slightly more potent, the new compound showed broad-spectrum antifungal activity without any resistance observed in tested strains like A. fumigatus or C. albicans. This suggests its promise as a potential antifungal candidate, especially for use in developing novel therapeutic agents.

L-Asparaginase-Producing Endophytic Bacteria in *Aegle marmelos*

Another study highlighted the presence of L-asparaginase-producing bacterial endophytes in A. marmelos, a discovery with important implications for cancer drug development. Optimization experiments revealed that the ideal conditions for enzyme production were:

Carbon source: Glucose

Temperature: 30°C

Incubation: 72 hours

Shaking speed: 120 rpm

pH: 8

The L-asparaginase activity correlated directly with the concentration of L-asparagine substrate. Two endophytic bacterial strains were identified as effective producers:

Strain 3: Klebsiella pneumoniae

Strain 4: Staphylococcus aureus

The results suggest the industrial potential of these isolates for large-scale L-asparaginase production, a key enzyme used in chemotherapy for treating acute lymphoblastic leukemia (Rathod *et al*., 2018).

Characterization of Endophytic Bacteria from *Aegle marmelos*

In a morphological and enzymatic characterization study, 15 bacterial isolates were obtained from *Aegle marmelos* leaves collected from three locations in Jabalpur, India. The isolates were analyzed on King's B medium, revealing the following morphological features:

60% of colonies: Irregular in shape

40%: Circular

73.33%: Flat elevation

26.67%: Raised

73.33%: Undulate margins

80%: Opaque, white colonies

Despite their diversity, none of the isolates exhibited detectable hydrolytic activity (i.e., no zones of clearance) for enzymes like amylase, cellulase, or protease, as tested on respective agar plates. This result suggests that these endophytes may have other, non-hydrolytic roles in the host plant's physiology or defense (Jain *et al*., 2021).

Enzyme and Phytochemical Production by Endophytic Fungi in the Western Ghats- A detailed study conducted in the Western Ghats of Tamil Nadu identified two dominant endophytic fungi from A. marmelos:

Alternaria citrimacularis (strain FC8ABr)

Curvularia australiensis (strain FC2AP)

These fungi were notable for producing a wide range of extracellular enzymes, including:Amylase,Protease,Lipase,Cellulase,Laccase, Xylanase

The fungi exhibited vigorous mycelial growth and enzyme production across a range of pH levels and incubation periods. Additionally, they synthesized phytochemicals like phenolics and flavonoids, both of which contributed to strong antioxidant activity. These metabolites likely play key roles in plant microbe interactions, including defense mechanisms and stress tolerance (Mani *et al*., 2018).

Discussion and Conclusion

Traditional healing systems, particularly Ayurveda, have long recognized the medicinal value of *Aegle marmelos*. Its therapeutic potential is attributed to a complex array of bioactive compounds such as coumarins, alkaloids, flavonoids, and terpenoids. Recent studies have brought attention to the critical role of endophytic microorganisms in both mimicking and enhancing the pharmacological effects of the host plant. Certain fungal endophytes Trichoderma harzianum, Aspergillus flavus, and Fusarium oxysporum have demonstrated noteworthy antioxidant, anticancer, and antidiabetic activities, due to their ability to produce secondary metabolites similar to those found in A. marmelos. These endophytes exhibit a broad spectrum of bioactivities, including antiviral effects against pathogens like SARS-CoV-2 and Newcastle Disease Virus, as well as the inhibition of enzymes such as xanthine oxidase and pancreatic lipase. Some even produce taxol-like compounds, underscoring their potential in cancer therapy.

Beyond their medicinal relevance, these microbial partners also contribute to industrial and environmental applications. Studies have demonstrated their use in green synthesis of nanoparticles and in enzyme production through solid-state fermentation, further highlighting the practical benefits of this plant-microbe association. Acting as alternative biofactories, endophytes not only expand the pharmacological repertoire of A. marmelos but also offer sustainable strategies to reduce pressure on overharvested or endangered medicinal plants. Their adaptability to controlled laboratory conditions makes them especially attractive for large-scale pharmaceutical development.

The identification of diverse endophytic fungi including genera such as Xylaria, Alternaria, Cladosporium, and Lasiodiplodia—through morphological and molecular tools reveals a rich and underexplored microbial ecosystem within the tissues of A. marmelos. Evidence suggests that tissue type and environmental factors significantly influence the colonization and metabolite production of these microbes. Combining modern microbiological and biochemical techniques with traditional knowledge reveals that *Aegle marmelos* is more than a medicinal plant—it is a reservoir of functionally diverse endophytes. These microorganisms, capable of reproducing or even enhancing the host plant's therapeutic properties, hold great promise as novel sources for drug discovery and development in the pharmaceutical industry.

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