Review Article

Dynamic Bioactive Potentials of Endophytes Inherent to *Aegle marmelos*: A Review

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Endophytes, which live asymptomatically within the healthy tissues of the host plant, have attracted researchers due to their massive bioactive potential. Some co-existing endophytes and their host plants have developed a unique interaction through time, which influences the creation of metabolic products in plants and thus the quality and amount of crude pharmaceuticals obtained from medicinal plants. Numerous investigations have shown that endophytes chemically produce secondary metabolites just like their hosts. Locals in Indian subcontinent and Southeast Asia have utilized *Aegle marmelos* for centuries to treat a variety of conditions, including dysentery, diarrhoea and dyspeptic symptoms. There have been numerous studies reporting the successful isolation of novel, beneficial bioactive compounds with antibiotic, anti-diabetic, antimicrobial and anti-cancer properties from endophytic fungi of this tree. The valuable bioactive chemicals produced by endophytic bacteria and actinomycetes have, however, received only a limited attention, making it a prime location for the discovery of new compounds for use in agricultural, and pharmaceutical industries. This review examines existing research data to reveal the biopotential of endophytes from *Aegle marmelos* as a promising source of naturally produced chemicals.

Key words: *Aegle marmelos*, bioactive, endophytic actinomycetes, endophytic bacteria, endophytic fungi, secondary metabolites

The link between man and his hunt for pharmaceuticals in nature extends back thousands of years, as evidenced by several sources including written records, preserved monuments and even original plant remedies. Popular perceptions of medicinal plant use and efficacy have a crucial role in the revelation of their therapeutic capabilities, resulting in their widespread prescription, even though their chemical constituents are not always fully understood. A medicinal plant is any plant that contains compounds that can be utilized for therapeutic purposes or the precursors for the production of valuable pharmaceuticals in one or more of its organs[1]. Traditional medicine is expected to be the primary source of health care for 80 % of the world's population[2], as these provide low-cost and safe health-care options in developing nations like India.

Aegle marmelos (*A. marmelos*) (FAMILY RUTACEAE)

A. marmelos also known as bael or golden apple is endemic to India and Southeast Asia. *A. marmelos* is a deciduous, medium-sized (12-15 m), slow-growing sharp tree with a short trunk, thick, soft, peeling bark and drooping lower branches[3]. Alkaloids, cardiac glycosides, coumarins, terpenoids, saponins, tannins, flavonoids, steroids, eugenol, lupeol, cineol, citronellal, cuminaldehyde, marmesin, auraptene, skimmianine, citral, luvangentin, anhydromarmelin, aegeline, marmesinine, marmelosin, marmelin, marmelide, psoralen, scopoletin, fagarine, limonene, citronellal, betulinic acid, imperatorin and cineole have been demonstrated to be present in parts of this tree[4-6].

Antimicrobial activity of *A. marmelos* has been widely studied[6-8]. The presence of linolenic acid and myristic acid in the leaf extracts of this tree

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has been associated with ameilioration of the risk of diabetes progression and improvement in insulin sensitivity[9,10]. *A. marmelos* was found to possess antitumor proliferative activity[11], and has also been shown to have antioxidant and antidyslipidemic properties[12,13].

MICROORGANISMS AS SOURCE OF BIOACTIVE COMPOUNDS

Microorganisms are important sources of bioactive natural products due to a number of characteristics, including their high surface area to volume ratio, rapid uptake of nutrients, high rates of metabolism and biosynthesis, adaptability to a variety of environmental conditions, and lack of stringent growth requirements[14].

In comparison to other terrestrial resources such as endophytic plants (3 %) and animal intestines (1 %), soil habitats (96 %) have been extensively studied[15]. Endophyte research had not been widely pursued until the discovery of *Taxomyces andreanae*, a taxol and taxane-producing endophyte isolated from *Taxus brevifolia*[16]. The term "endophyte" refers to a type of microorganisms that lives in the intracellular or intercellular areas of healthy plant tissues for part or all of their lives, with no evident symptoms or harmful consequences on their plant hosts[17].

Endophytes have adapted to niches and created a perfectly compatible symbiotic relationship *via* gene control over a long period of co-evolution with host plants[18]. These microorganisms can strengthen their hosts' resilience to biotic (pathogens, herbivores, insects, etc.) and abiotic (drought, flood, high salt, incorrect temperature, etc.) conditions[18-20]. Endophytes are also capable of secreting some bioactive compounds that can successfully inhibit the prevalence of tuberculosis, malaria, arthritis, diabetes and autoimmune diseases[21-25].

Endophytic microbes from many Indian medicinal plants have remained undiscovered despite their enormous biological potential, prompting further research in this area[26]. Due to their chemical contents, medicinal and aromatic plants are assumed to harbour only a few microorganisms that can develop and survive in the presence of these substances[27]. As a result, endophytic microbes may be able to produce substances that resemble the qualities of *A. marmelos*.

DIVERSITY OF ENDOPHYTIC MYCOFLORA IN *A. marmelos*

A total of 25 studies discussed endophytic fungi in relation to *A. marmelos*. Seven studies related to bacterial endophytes were obtained. Only 1 study described presence of actinomycetes in endophytic form in *A. marmelos*. A total of 28 articles discussed the isolation procedure from various organs, *viz.,* root, branch, stem/twig, bark, leaf and fruit (Table 1). Most of the isolates were found to be fungal strains (n=511, Table 1). The isolates were obtained from root, stem, twig, branch, bark, and leaf tissues, using various media like Potato Dextrose Agar (PDA), King's B (KB), Malt Extract Agar (MEA), Sabouraud's Dextrose Agar (SDA) and Water Agar (WA).

Most commonly recovered stem endophytic fungal genera included *Chaetomium*, *Botryosphaeria*, *Fusarium*, *Pestalotiopsis*, *Pheoacremonium* and *Pheoacremonium*[28,29]. As per Gawas et al.[27], *Aspergillus fumigatus* and *Aspergillus niger* colonized the bark, *Vermiculariopsiella parva* colonized the leaf while a non-sporulating morphotype colonized the stem. The variable number of endophytic fungal species existing in various tissue types indicates that no single tissue harbors richer endophytic diversity than the other, and that endophytes exhibit strict tissue preference. *Diaporthe* sp. was the most frequently isolated endophyte during the dry season while *Ophioceras commune* was more frequently isolated during the wet season. It was observed that stem tissue was maximally colonized in both the seasons. Overall tissue colonization was more in the wet season as compared to the dry and average colonization under different conditions was 82 %[27].

A total of 44 endophytic bacteria were found to be isolated from *A. marmelos*. Out of these, 34, 4 and 2 isolates were obtained from leaves, stem and petiole respectively. Media used for isolation included Tryptic Soy Agar (TSA), KB and Nutrient Agar (NA). The bacterial genera identified were as follows; *Aeromonas*, *Azomonas*, *Bacillus*, *Corynebacterium*, *Klebsiella*, *Kosakonia*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, etc. Interestingly, only 1 study demonstrated the presence of bacteria in *A. marmelos* fruit (n=4). In this study NA was used to recover Gram positive bacteria from fruit[30]. An actinomycete *Microbispora* sp. was reported to be an endophyte

of *A. marmelos*[31]. Although this tree species is native to Indian subcontinent and Southeast Asia[32], the studies related to its endophytic microbiota were found to be limited to Myanmar[3] and India.

BIOLOGICAL ACTIVITIES OF *A. marmelos* ENDOPHYTES

Antagonistic potential:

Endophytic microbes from *A. marmelos* demonstrated a broad range of antibacterial activity against both Gram positive and Gram negative bacteria. The antibacterial activity by various cultures, spent broths and crude/partially purified/Ethyl Acetate (EA) extracts was found to range between 0-26 mm (Table 2). As per Meshram *et al.*[19], the Volatile Organic Compounds (VOCs) produced by *Muscodor kashayum* showed a broad-spectrum antimicrobial activity against human bacterial pathogens namely *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and completely inhibited their growth after an exposure for 48-72 h.

Remarkable antagonistic activity (100 % inhibition) was exhibited by *Muscodor kashayum* against *Bionectria*, *Cercospora*, *Chaetomium*, *Colletrotrichum*, *Curvularia*, *Fusarium*, *Lasiodiplodia*, *Mycosphaerella*, *Penicillium*, *Rhizoctonia*, *Trichoderma*, *Agaricus*, *Pleurotus* and *Candida* (Table 2). Various bacterial and fungal isolates inhibited *Candida albicans* and *Aspergillus* sp.[19,26,30,33].

TABLE 1: ENDOPHYTES REPORTED FROM VARIOUS TISSUES OF *A. marmelos*

Domain	Author	Isolation media	Source tissue: isolates	Species studied
Bacteria	Vichare *et al.*[49]	NA	Leaf: 2	*Bacillus* sp.
	Rathod *et al.*[41]	TSA	Leaf: 4	*Staphylococcus aureus*, *Klebsiella* pneumoniae
	Panigrahi *et al.*[20]	NA	04:Leaf (1), stem (2), petiole (1)	*Bacillus* sp., *Pseudomonas* sp.
	Myint[3]	NA supplemented with 1% glucose/ sucrose/lactose	Leaf: 11	*Bacillus*, *Streptococcus*, *Azomonas*, *Corynebacterium*, *Pseudomonas*, *Aeromonas*
	Londhe *et al.*[30]	NA	Fruit: 4	Gram positive bacteria
	Jain *et al.*[61]	KB	Leaf: 15	NR
	Panigrahi *et al.*[52]	NA	04:Leaf (1), stem (2), petiole (1)	*Kosakonia cowanii*
Fungi	Prabhavathy *et al.*[62]	PDA	Leaf: 1	*Rhizopus* sp.
	Gawas *et al.*[27]	MEA (2%)	28:00:00	*Diaporthe* sp., *Ophioceras commune*, *Lasmeniella* sp., *Phomopsis arnoldiae*, *Aspergillus fumigatus*, Eurotium rubrum, *Fuckelia ribis*, *Vermiculariopsiella parva*,
			Leaf (9), stem (11), bark (7), stem-bark overlap (1)	*Aspergillus niger*, *Colletotrichum gloeosporioides*, *Phomopsis stipcita*, etc.
	Patil *et al.*[26]	WA	Leaf: 1	*Aspergillus flavus* L7
	Patil *et al.*[40]	PDA	Leaf and bark: 12	NR
	Meshram *et al.*[29]	PDA	Stem: 48	*Lasiodiplodia pseudotheobromae*, *Alternaria* sp., *Aureobasidium* sp., *Botryosphaeria* sp., *Fusarium* sp., *Pestalotiopsis* sp., *Neofusicoccum parvum*, *Togninia* sp., *Pheoacremonium* sp.
	Meshram *et al.*[19]	PDA	NR: 1	*Muscodor kashayum*
	Mani *et al.*[45]	PDA, MEA and SDA	NR: 2	*Curvularia australiensis*, *Alternaria citrimaculairs*

	Mani *et al.*[47]	PDA, MEA and SDA	Root, inner stem, inner branch and leaf: 169	*Curvularia* sp., *Alternaria* sp., *Cladosporium* sp., *Aspergillus* sp.
	Kumari *et al.*[18]	PDA	Leaf: 1	*Fusarium* sp.
	Krishnamurthy *et al.*[28]	PDA	NR: 20	*Ascomycota*, sterile isolates
	Gond *et al.*[60]	PDA	Leaf: 1	*Phoma herbarum*
	Badiya *et al.*[44]	Not applicable*	Not applicable*: 23	*Botrytis cinera*, *Trichoderma harzianum*, *Phoma* sp., *Hyalopus* sp., *Fusarium* sp., *Aerophiolophora fusispora*, *Penicillium janthinellum*, *Masoniella*, *Fusarium* monoliforma, *Phoma chrysanthemicola*, Hülle cells, *Phomopsis* sp., *Thamnidium* sp., *Aspergillus oryzae*
	Gond *et al.*[63]	PDA	Inner bark, leaf and root: 79	*Fusarium* spp., *Aspergillus* spp., *Alternaria* sp., *Drechslera* sp., *Rhizoctonia* sp., *Curvularia* sp., *Nigrospora* sp., *Stenella* sp., *Chaetomium globosum* and *Emericella* sp. (perfect state of *Aspergillus* sp.)
	Kapoor *et al.*[37]	PDA	Stem and leaf: 14	*Lasiodiplodia pseudotheobromae*, *Lasiodiplodia* sp., *Fusarium* sp., *Muscodor* sp.
	Mani *et al.*[33]	PDA, MEA and SDA	Bark, branch and leaf: 16	*Aspergillus terreus*
	Arora *et al.*[32]	WA	Leaf: 1	*Xylaria psidii*
	Meshram *et al.*[64]	PDA	Leaf, stem and internal stem: 25	*Fusarium* sp., *Aureobasidium* sp., *Alternaria* marmelos, *Lasiodiplodia* sp., *Sphaeropsis* sp., *Barriopsis* sp., *Cunninghamella* sp., *Penicillium* sp., Mycelia-sterilia
	Gupta *et al.*[39]	PDA	Leaf, stem, bark: 52	*Fusarium incarnatum*, *F. commune*, *F. chlamydosporum*, *F. culmorum*, *F. equiseti*, *F. moniliforme*, *F. solani*, *F. oxysporum*, *F. semitectum*, *Botryosphaeria* sp., *B. stevensii*, *Barriopsis iraniana*, *Diplodia* sp., *Lasiodiplodia pseudotheobromae*, *L. theobromae*, *L. gonubiensis*, *Sphaeropsis sapinea*, *Aspergillus alternata*, *A. solani*, *A. marmelos*, A. niger, *Penicillium chrysogenum*, *Trichoderma viride*, *M. kashayum*, *P. microspora*, *P. rubrigenum*, *Phomopsis* sp, *Aureobasidium* sp.
	Gangadevi *et al.*[34]	KB	Leaf: 1	*Bartalinia robillardoides* Tassi
	Kapoor *et al.*[38]	Not applicable*	Not applicable*: 7	*Muscodor* sp.
	Patil *et al.*[48]	NR	Not applicable*: 1	*Aspergillus flavus* L7
	Sharma *et al.*[36]	MEA	NR: 1	*Alternaria* sp.
	Subramanian *et al.*[58]	PDA	Leaf: 3	*Colletotrichum gloeosporiodes*
	Vellingiri *et al.*[59]	Not available	Not available: 1	*Aspergillus terreus* FC36AY1
	Meshram *et al.*[43]	WA	Twigs: 3	*Fusarium* equiseti (*Fusarium* sp. NFCCI 2946 and *Fusarium* sp. NFCCI 2904), *Fusarium incarnatum* (*Fusarium* sp. NFCCI 2905)
Actinomycetes	Saini *et al.*[31]	Not applicable*	Not applicable*: 1	*Microbispora* sp.

Note: NR: Not Reported; Not applicable*: No isolation performed; NA: Nutrient Agar; PDA: Potato Dextrose Agar; KB: King's B; MEA: Malt Extract Agar; SDA: Sabouraud's Dextrose Agar; WA: Water Agar

TABLE 2: BIOACTIVE PROPERTIES AND COMPOUNDS FROM ENDOPHYTIC BACTERIA, FUNGI AND ACTINOMYCETES BELONGING TO *Aegle marmelos*

Author	Species antagonized (zone of inhibition in mm)	Other bioactive properties	Bioactive compounds identified
Vichare *et al.*[49]	NR	NR	Total phenols 12.83 and 11.33 µg/ml in liquid medium
Rathod *et al.*[41]	NR	NR	L-asparaginase
Panigrahi *et al.*[20]	*S. aureus* and *E. coli* (11-15)	P-solubilization	IAA, siderophore
Myint[3]	*V. cholerae*, *P. aeruginosa*, *S. aureus*, *K. pneumonia*, *P. mirabilis*, *C. albicans*, *E. coli* and *B. subtilis* (0-24.6)	NR	NR
Londhe *et al.*[30]	*E. coli*, *K. pneumoniae*, *S. aureus* and *B. subtilis* (0-20), *C. albicans* (22), *Aspergillus* sp. (25)	NR	Phenol, terpenoids and tannins in culture supernatants
Panigrahi *et al.*[52]	NR	P-solubilization	Oxalic acid, malic acid, tartaric acid, gluconic acid
Prabhavathy *et al.*[62]	*P. aeruginosa* (12)	NR	NR
Patil *et al.*[26]	*E. coli*, *P. aeruginosa*, *Salmonella abony*, *S. typhi*, *B. subtilis* and *S. aureus* (EA extract: 10-18), *Penicillium citrinum*, *A. niger*, *C. albicans* (EA extract: 15-23)	DPPH-free radical scavenging effects of 64.53 % (700 µg/ml), membrane stability	65.85±0.49 mg GAE/ml in the culture filtrate
Patil *et al.*[40]	NR	NR	Asparaginase
Meshram *et al.*[29]	NR	Fibrinolytic and proteolytic activities	NR
Meshram *et al.*[19]	*S. aureus*, *E. coli* and *P. aeruginosa* (100% inhibition), *Bionectria*, *Cercospora*, *Chaetomium*, *Colletrotrichum*, *Curvularia*, *Fusarium*, *Lasiodiplodia*, *Mycosphaerella*, *Penicillium*, *Rhizoctonia*, *Trichoderma*, *Agaricus*, *Pleurotus*, *C. albicans* (100% inhibition), *A. flavus* (16), *A. alternata* (17)	NR	3-cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (synonymn : beta- Bisabolol), 2, 6-bis-(1,1-dimethylethyl)-4-(1-oxopropyl) phenol, 1,6-dioxacyclododecane-7,12-dione, 2, 3-dihydro-1,1-dimethyl-6- tert-butyl-1H-indene-4-acetic acid, 2,4-di-tert-butylthiophenol and 4-octadecylmorpholine, and some unidentified volatiles
Mani *et al.*[45]	NR	NR	GAE (15.5 and 20.0 µg/mg of the extract), enzymes (amylase, protease, cellulase, lipase, xylanase), phenols, flavonoids, tannins, cardiac glycosides, steroids, saponins, alkaloids
Mani *et al.*[47]	*S. aureus*, *S. epidermidis*, *E. faecalis*, *Shigella* sp., *E. coli*, *P. aeruginosa*, *K. pnuemoniae*, *S. typhi* and *P. mirabilis* (Not Available)	Reducing power, metal chelating activity, DPPH-free radical scavenging effects (IC50=43-200 µg/ml)	NR
Gond *et al.*[60]	*Shigella flexnii*, *Shigella boydii*, *S. enteritidis*, *S. paratyphi*, *P. aeruginosa* and *Morganella morganii* (column chromatography fractions: <10 to >20)	NR	1-iodo-naphthalene
Badiya *et al.*[44]	NR	NR	Cellulase
Kapoor *et al.*[37]	NR	Xanthine oxidase inhibitory activities	NR

Mani *et al.*[33]	*S. typhi, S. aureus, S. epidermidis, E. coli, P. aeruginosa, K. pnuemoniae, V. cholerae* and *P. mirabilis* (0-24), *C. albicans* (0-24), *A. niger* (0-22)	Synthesis of CuO nanoparticles for antagonistic, anticancer and free radical scavenging properties, reductive power and angiogenesis inhibition	NR
Arora *et al.*[32]	NR	Cytotoxic potential	Xylarione A, (-) 5-methylmellein
Meshram *et al.*[64]	*S. aureus* (spent broth: 0-15, EA extract: 0-26)	NR	NR
Gupta *et al.*[39]	NR	Lipase inhibitory activity	Terpenoids, carbohydrates, amino acids
Gangadevi *et al.*[34]	NR	Anticancer activity	Taxol
Kapoor *et al.*[38]	NR	Xanthine oxidase inhibition, reduction in uric acid production, DPPH free radical scavenging property (46.4 %-71.6 %)	NR
Patil *et al.*[48]	NR	NR	Flavonoid quercetin (158.33 mg/ml of crude extract); HPTLC peak for standard rutin and for L7 extract at Rf 0.320.02
Sharma *et al.*[36]	NR	Alpha-glucosidase inhibitory activity	NR
Subramanian *et al.*[58]	NR	Copper nanoparticles synthesis	NR
Vellingiri *et al.*[59]	NR	Silver nanoparticles synthesis, angiogenesis inhibition	NR
Meshram *et al.*[43]	NR	Proteolytic and fibrinolytic activities	NR
Saini *et al.*[31]	NR	Alpha-amylase and alpha-glucosidase inhibitory potentials, EA extract: Hydroxyl radical scavenging activity (IC_{50}=18.91µg/ml), nitric oxide scavenging activity (IC_{50}=2330.47µg/ml), superoxide scavenging effects (IC_{50}=10183.06 µg/ml), reducing power	EA extract: Total phenolic compounds: catechol eq. (42.11±1.88 mg/g of the extract) and GAE (6.47±0.95 mg/g of the extract).

Note: NR: Not reported; IAA: Indole-3-Acetic Acid; EA extract: Ethyl Acetate extract; GAE: Gallic Acid equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl

ANTICANCER ACTIVITY

The last decade has seen a surge in interest in finding fungi that produce anti-cancer compound taxol. Gangadevi *et al.*[34] reported that endophytic fungus *Bartalinia robillardoides* Tassi, from *A. marmelos* Correa ex Roxb produced 187.6 µg/l taxol in liquid culture. Apoptotic cell death was accelerated by taxol doses (0.005-0.05 µM) and was found to be cell type dependent in various human cancer cell lines (H116, BT220, HL251, Int407 and HLK210).

A fungal endophyte isolated from *A. marmelos* leaves, *Xylaria psidii*, was tested for cytotoxicity. The half maximal Inhibitory Concentration (IC_{50}) range of 16±1.09 to 25±1.98 µM was determined in different human cancer cell lines (MCF-7, MIA-Pa-Ca-2, NCI-H226, HepG2, DU145). Fungal metabolites Xylarione A and (-) 5-methylmellein induced concentration dependent apoptotic cell morphology in Mia-Pa-Ca-2 cells, including nuclear condensation, membrane blebbing and apoptotic body evolvement. These compounds were also associated with a widening of mitochondrial permeability transition pores resulting in the leakage of proapoptotic proteins from mitochondria to cytosol[32].

ENZYME INHIBITORY ACTIVITIES

Inhibition of amylolytic enzymes:

Diabetes-induced chronic hyperglycemia causes

long-term organ damage, dysfunction and eventually failure, causing peripheral vascular disease, nephropathy, neuropathy, retinal damage, morbidity and mortality[35]. Rapid degradation of dietary starch by enzymes like α-amylase and α-glucosidase leads to postprandial hyperglycemia. Thus, slowing starch digestion by inhibiting enzymes would help control diabetes.

Only two investigations indicated that endophytes associated with *A. marmelos* inhibited amylolytic enzymes. Sharma *et al.*[36] reported the inhibition of α-glucosidase by EA extract of an *Alternaria* sp. endophyte (IC_{50}=763 µg/ml). Likewise, Saini *et al.*[31] investigated the *in vitro* enzyme inhibitory activities of an endophytic actinomycete EA extract. A higher ability to overcome postprandial glucose concentrations was shown by the low IC_{50} values for α-amylase and α-glucosidase (1950.71 and 391.38 µg/ml, respectively).

Xanthine oxidase inhibition:

Hyperuricemia (a risk factor for gout), is a metabolic disorder characterized by an increased serum urate levels (>6-7 mg/dl). One of the strategies for treatment of hyperuricemia involves blocking Xanthine Oxidase (XO), the principal enzyme involved in uric acid formation. Only Allopurinol and Febuxostat have been clinically validated as oral XO inhibitors, respectively, for the treatment of hyperuricemia and gout. Kapoor *et al.*[37] studied culture filtrates of 9 endophytic fungi for XO inhibitory activity. The activity displayed by the *Lasiodiplodia pseudotheobromae* isolate (54.8 %) was higher than allopurinol (44.2 %) and on par with Febuxostat (55.8 %). Other isolates with XO inhibitory property were *Fusarium* (6 isolates, range: 17.6 %-44.2 %), *Muscodor* (1 isolate, 37.1 %) and unidentified (2 isolates, 17.6 %, 20.3 %). In another study, Kapoor *et al.*[38] reported that crude chloroform fractions of 7 *Muscodor* sp. isolates displayed XO inhibition within a range of 28.6 % to 91.4 %. The inhibitory activity of *Muscodor darjeelingensis* (91.4 %) was higher than Allopurinol (88.0 %), the reference drug.

Lipase inhibitory activity:

Pancreatic Lipase (PL) is a crucial enzyme in lipid metabolism that helps to prevent triglyceride production. Orlistat is a Food and Drug Administration (FDA) approved drug with adverse effects like oily stools, gas, faecal urgency and abdominal cramps. This has prompted researchers to look for novel sources of PL inhibitors that are both safe and effective. Using twigs from *A. marmelos*, Gupta *et al.*[39] isolated 52 endophytic fungal isolates. The porcine PL qualitative plate assay was tested positive by culture filtrates from 34 isolates. Rhodamine and Phenol red olive oil plate assays displayed inhibition in the range of 4.49 %-96.57 % and 0.00 %-96.52 %, respectively. Quantitative examination of *Fusarium incarnatum* (leaf isolate), *Botryosphaeria stevensii* and *Fusarium semitectum* (stem isolates) culture filtrates, using p-nitrophenyl laurate as the substrate confirmed the qualitative results, indicating 96.7 %, 85.07 % and 80.14 % inhibition, respectively. The aqueous extract of *Fusarium incarnatum* had a lower IC_{50} (2.12 µg/ml) than the positive control orlistat (IC_{50}=2.73 µg/ml). Similarly, the IC_{50} of *Botryosphaeria stevensii* and *Fusarium semitectum* crude EA extracts were 14.48 µg/ml and 28.18 µg/ml, respectively. These findings substantially support the hypothesis that endophytic microbes mimic the anti-PL properties of the host tree *A. marmelos* and should be further tested.

ENZYME PRODUCTION

Endophytes from medicinal plants are thought to inhabit unusual and severe habitats, which may contain novel metabolic and enzymatic processes that help protect their hosts and help them build new colonies. Endophytes are thus, the most potent means of identifying new enzymes for medical usage.

Asparaginase synthesis:

The L-asparaginase enzyme has been employed in tumor therapy as part of a combination therapy approach. To uncover asparaginase producing filamentous fungi, Patil *et al.*[40] developed a dye-based agar plug method, supplementing the Czapek Dox agar medium with phenol red. Agar plugs were transferred to glass slides in a Petri dish with Whatmann filter paper no. 1.

The fungal spore suspensions were used to inoculate the agar plugs, followed by incubation of Petri dishes in a humidity chamber at 28° for 4 d. All 12 endophytic fungi isolates tested were able to grow on modified agar medium. The color intensity was shown to be directly proportional to

asparaginase activity.

According to Rathod *et al.*[41], the maximum concentrations of L-asparaginase produced by *Klebsiella* pneumoniae and *Staphylococcus aureus* (isolated from *A. marmelos* leaves) were found to be 2.75 IU/ml and 2.68 IU/ml, respectively. The optimization tests determined that the maximum amount of enzyme could be produced at 30° for 72 h in a 120 rpm rotatory shaker incubator with a pH of 8. Glucose was discovered to be the most appropriate carbon source.

Fibrinolytic activity:

Fibrin is the most critical protein in blood clotting. Thrombolytic therapy uses intravenous streptokinase and urokinase infusions to swiftly restore blood flow blocked by fibrin, reducing morbidity and death[42]. However, lack of specificity for fibrin, internal bleeding, short half-life and low economic viability are some of the downsides.

Preliminary screening of the culture filtrates from *A. marmelos* endophytic fungal isolates led to halo formation on plate (range=63.58-113.04 mm^2), with highest fibrinolytic activity been reported for *Lasiodiplodia pseudotheobromae*[29]. Subsequent ammonium precipitation and dialysis revealed a protein (80 kDa) with a specific fibrinolytic activity of 3.56 U/ml. Meshram *et al.*[43] also reported fibrinolytic activity by 2 isolates, *Fusarium* sp. NFCCI 2904 (94.98 mm^2) and *Fusarium* sp. NFCCI 2905 (125.81 mm^2).

Other enzymes:

Protease activity was found to range between 31-191 mm^2 by various fungal isolates[29,43]. Badiya *et al.*[44] found that the endophytic fungus *Trichoderma harzianum* produced more enzymes (cellulase, endo-β-1,4-glucanase and β-glucosidase) in a Solid State Fermentation (SSF) environment than in a Submerged Fermentation (SmF) environment. *T. harzianum* had a significantly higher filter paper assay-based cellulase activity (4.27 FPU/ml) on the 10^{th} d. The maximum enzyme activity of endo-β-1,4-glucanase was observed on the 6^{th} d (18.38 IU/ml). The isolate produced 17.00 IU/ml of β-glucosidase on both the 6^{th} d and 10^{th} d under SSF conditions. As per Mani *et al.*[45], release of most of the enzymes (amylase, xylanase, protease and cellulase) was favoured by neutral pH. On the other hand, lipase production by *Curvularia australiensis* peaked at pH 9.

FREE RADICAL SCAVENGING ACTIVITY

Free radicals have been connected to the development of several diseases, including atherosclerosis, cancer, diabetes and liver cirrhosis, among others, and compounds that can scavenge free radicals have a high possibility for amendment of these disease processes. Saini *et al.*[31] reported the free radical scavenging potentials of a *Microbispora* sp. EA extract against nitric oxide, superoxide and hydroxyl radicals (Table 2). At all concentrations tested (100, 250, 500, 750 and 1000 µg/ml), the extract produced at least 60 % scavenging effects against hydroxyl radicals. Likewise, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging potentials of endophytic microorganisms from *A. marmelos* have been widely demonstrated (Table 2).

The endophytes' free radical scavenging activity reveals that they have the ability to donate hydrogen to free radicals, thereby eliminating the odd electron and serving as an antioxidant in the process. These antioxidants have the ability to suppress free ions through chelating actions, thus assisting in the management of Cu^{2+} and Fe^{2+} levels without causing cell harm[46]. Mani *et al.*[47] demonstrated that endophytic *A. marmelos* fungal extracts inhibited the formation of ferrous and ferrozine complexes, implying the presence of metal chelating activity. *Alternaria citrimacularis*, *Alternaria alternata*, *Curvularia cladosporioides*, *Curvularia australiensis* and *Aspergillus niger* had IC_{50} values of 66, 156, 181, 50 and 178 µg, respectively. In this regard, *Pleosporales* isolates (*Curvularia australiensis* and *Alternaria citrimacularis*) possessed a stronger chelating capacity, allowing them to efficiently capture free radicals at low concentrations.

Compounds with reducing power act as electron donors and can reduce oxidized intermediates in lipid peroxidation processes, enabling them to act as primary and secondary antioxidants[46]. Reducing power of the endophytic fungi and actinomycete was found to increase with increasing concentration[31,47], as suggested by their absorbances (*Alternaria alternata*, *Alternaria citrimacularis*, *Curvularia australiensis*, *Curvularia cladosporioides* and *Aspergillus niger*, range=0.1-0.9, *Microbispora* sp., range=0.024-0.032).

The most abundant phytochemicals found in plants and plant products, phenolics can be classified into several subgroups based on their

chemical structures, including phenolic acids, flavonoids, tannins, coumarins, curcuminoids, lignans, quinones and stilbens. Several workers reported the production of phenols by *A. marmelos* endophytes[26,30,48,49]. Flavonoids are considered to be the strong scavengers of Reactive Oxygen Species (ROS). Mani *et al.*[45] outlined that the total flavonoids present in hexane and EA extracts of *Curvularia australiensis* were described to be 22.2 µg rutin/mg and 89.5 µg rutin/mg of the extract, respectively. This obtained value was higher when compared to the extract of *Alternaria citrimaculairs* (hexane, petroleum ether and methanol-64.0, 21.6 and 42.5 µg rutin/mg of the extract). Other phytochemicals identified from the extracts included cardiac alkaloids, glycosides, saponins, steroids and tannins[45].

PLANT GROWTH PROMOTING ACTIVITIES

Phosphate solubilization:

Secretion of low molecular weight organic acids by endophytic microbes help them release bound phosphate by inhabiting the soil particle's P adsorption site[50,51]. Panigrahi *et al.*[20] reported that isolate AP01 exhibited a Phosphate Solubilizing Index (PSI) of 3.18. Likewise, *Kosakonia cowanii* obtained by Panigrahi *et al.*[52] had a PSI of 4.5 in Pikovaskaya agar and exhibited maximal phosphate solubilization in National Botanical Research Institute's (NBRI) broth (70.20 µg/ml) on 4th d at pH 3.6. High Performance Liquid Chromatography (HPLC) identified the release of oxalic, malic, tartaric and gluconic acids into the medium[52].

Siderophore production:

Siderophore synthesis by microorganisms results in the uptake of metals such as iron, zinc, copper and other elements by plants. AP01, a bacterial isolate obtained from petiole of *A. marmelos*, demonstrated a high level of efficiency in the formation of siderophore, as measured by the siderophore production index of 2.39[20].

Indole-3-Acetic Acid (IAA) Production:

It has also been demonstrated that endophytes enhance plant growth by releasing phytohormones such as IAA, which not only result in increased root size, but also facilitate greater nutrient absorption by the plant. Panigrahi *et al.*[20] quantified 13.13 µg/ml of IAA production by AL01, an endophytic bacterium recovered from leaf tissues of *A. marmelos*.

MEMBRANE STABILITY

During inflammation, lysosomal hydrolytic enzymes are secreted, causing organelle damage and abnormalities. Various methods have been used to investigate anti-inflammatory compounds, utilizing methods such as inhibiting protein denaturation, stabilizing erythrocyte/lysosomal membranes, fibrinolytic tests, platelet aggregation, etc. Patil *et al.*[26] compared membrane-stabilizing characteristics of the endophytic *Aspergillus flavus* A7 to Ibuprofen, the standard drug. The RBC hemolysis inhibition of the EA extract was practically similar to Ibuprofen at 1.8 mg/ml, showing high potential for the former. Total flavonoid content in the culture filtrate was found to be 158.33 mg quercetin/ml of crude extract, which has been associated with lysosomal membrane stabilization[53].

NANOPARTICLES SYNTHESIS

Myco-nanotechnology has been widely accepted due to quick growth, simple structures and high biomolecule production by fungi, which stimulate the synthesis of Nanoparticles (NPs). The ability of fungi to create metallic and oxide NPs such as gold, silver, copper, titanium and zinc has been studied in detail[54,55].

Copper nanoparticles:

The antimicrobial, antioxidant, quorum quenching, anticancer, antiviral, anti-helminthic, anti-parasitic, catalytic and anti-insect effects of copper and its oxide forms has motivated researchers to synthesize copper and copper oxide nanoparticles (CuNPs and CuONPs). The CuONPs from endophytic *Aspergillus* terreus FCBY1 exhibited the antagonistic potential against human clinical pathogens (Proteus mirabilis, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Aspergillus niger* and *Candida albicans*) in a range of 9-23 mm. The particles also demonstrated free radical/ROS scavenging at low concentrations, as measured by scavenging of DPPH (IC_{50}=0.080 mg/ml) and nitric oxide radicals (IC_{50}=0.096 mg/ml). 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl

tetrazolium bromide (MTT) (IC_{50}=22 µg/ml) and Fluorescence-Activated Cell Sorting (FACS), demonstrated their anticancer activities in colon cancer cells (HT-29, 32.11 % cells gated in S phase of cell cycle). *In vivo* Hen's Egg-Chorioallantoic Membrane (HET-CAM) testing revealed that a 60 µl dose blocked blood vessel growth by 81.81 % (after 18 h) in tumor cells, thus exhibiting control of angiogenesis[33].

Nonetheless, using CuONPs introduces structural instability and physiologic difficulties such as severe effects on the central nervous system as well as leads to respiratory dysfunction[56,57]. Use of chitosan polymer and graphene oxide can prove effective in these regards. The CuNPs for conjugation with Graphene Oxide Conjugated Chitosan (GO-CS) were synthesized using the potential leaf endophytic fungal isolate *Colletotrichum gloeosporiodes* A212[58]. CuNPs/GO-CS demonstrated antibacterial activity against *Escherichia coli* MTCC 443 with a 5 mm zone of inhibition. At 24 h, the IC_{50} values were determined to be 1000 µg/ml for VERO and 15 µg/ml for MCF7, using MTT cytotoxicity assay. The inhibition ratio of CuNPs/GO-CS was 25 %. Morphological analysis of VERO and MCF7 cells treated for 24 h with 15 µg/ml of CuNPs/GO-CS revealed a dramatic phenotypic change leading to cell death.

Silver nanoparticles:

Vellingiri *et al.*[59] utilized endophytic *Aspergillus terreus* FC36AY1 to produce Silver Nanoparticles (AgONPs). The antibacterial and antioxidant properties of the myco-generated AgONPs were demonstrated, with the maximum activity observed at the lowest concentration. Furthermore, the suppression of angiogenesis by the AgONPs in the Chittagong breed Hen's Egg Test on the Chorio-Allantoic membrane revealed significant bioactivity even at the lowest concentration of 0.1 µg/ml, on the Chorio-Allantoic membrane.

OTHER BIOACTIVE COMPOUNDS

Various other extracellular biological metabolites were identified from endophytic microorganisms. Most of these included inorganic compounds like 3-cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (synonymn: beta- Bisabolol, 2, 6-bis-(1,1-dimethylethyl)-4-(1-oxopropyl) phenol, 1,6-dioxacyclododecane- 7,12-dione, 2, 3-dihydro-1,1-dimethyl-6- tert-butyl-1H-indene-4-acetic acid, 2,4-di-tert-butylthiophenol, 4-octadecylmorpholine and 1-iodo-naphthalene[19,60]. However, organic molecules like terpenoids, carbohydrates, amino acids, oxalic acid, malic acid, tartaric acid and gluconic acid, were also identified[39,52].

SUMMARY AND PROSPECTIVES

A. marmelos is one of these trees whose therapeutic properties have been utilised and well acknowledged since ancient times. Recent shifts in the focus of researchers toward endophytes have resulted in a plethora of literature on the essential characteristics of endophytes on this tree. Numerous fungi, bacteria, and actinomycetes that inhabit the tree have been extensively researched for their bioactive properties. Endophytes of *A. marmelos* are renowned for their antibacterial, anticancer, plant growth-promoting, metabolite-producing, enzyme-inhibiting, and antioxidant properties. In addition, a surge has been seen in antimicrobial, antioxidant, anticancer potentials of these endophytes, with the NP-synthesis technology. Despite the widespread knowledge of the benefits of *A. marmelos* endophytes, there is a dearth of literature and pharmaceuticals based on these resources.

There is a need to delve more into the nuances of the microbial diversity found in this tree species. The majority of the research has been undertaken in India. There is potential for recovering useful microorganisms from different regions of the world, which may exhibit more diversity in endophyte taxa and have more diversified bioactive characteristics. Moreover, most of the studies are *in vitro* and thus, do not confirm the suitability of these metabolites in human/animal models. Further studies are necessary to confirm the safety of bioactive metabolites from *A. marmelos* endophytic extracts in living systems. Nausea, abdominal discomfort, bloating, diarrhoea, liver toxicity, bleeding, as well as unsuitability for pregnant/geriatric populations are some of the concerns with existing drugs, which must be studied in detail.

Therefore, the purpose of this study was to investigate in depth and bring to the notice of the scientific community the applications and benefits associated with this native and easily cultivable

tree. Present review comprehensively highlights the potentiality of endophytic microbiota from *A. marmelos* as an effective choice for pharmaceutical industry and agriculture. Moreover, there is ample room for future research, particularly in the realm of nanoparticle synthesis, in unexplored fields (bacteria and actinomycetes). The use of chemical components of *A. marmelos* for the treatment of various diseases can pave the way for the creation of useful therapeutic medicines.

Conflict of interest:

The authors declared no conflict of interests.

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