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Review Article

Bio-Metabolomic odyssey: Deciphering the antimicrobial armamentarium of *Endophytic Fungi* in *Anogeissus latifolia* and related Sps. for therapeutic advancements

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ABSTRACT: The review presents a comprehensive overview of the potential therapeutic advancements facilitated by microbial metabolites, particularly focusing on the symbiotic relationship between endophytic fungi and *Anogeissus latifolia* and related species. These fungi produce a diverse array of metabolites with pharmacological significance, including antibacterial, anticancer, antioxidant, and anti-diabetic properties. The review highlights the importance of endophytic fungi in natural drug discovery, emphasizing their role in enhancing plant resilience and producing therapeutically relevant compounds. Chemical analysis of A. latifolia reveals compounds like ellagic acid derivatives and flavonoids with medicinal potential, while pharmacological investigations showcase the plant's efficacy in traditional applications and its promising antibacterial properties. Methodologies for identifying and characterizing bioactive compounds, such as metabolomics and high-performance thin chromatography, are outlined, supporting the hypothesis that undiscovered bio-metabolites from endophytic fungi hold high antibacterial potential, thus offering new avenues for drug discovery. In conclusion, the exploration of microbial metabolites, particularly those derived from endophytic fungi in A. latifolia, presents promising opportunities for developing novel, environmentally friendly, and effective therapies. This review underscores the significance of A. latifolia in herbal medicine and drug discovery, emphasizing the importance of preserving biodiversity and sustainable resource utilization.

KEYWORDS: bio-metabolites; pharmacological insights; medicinal plants; endophytic fungi; therapeutic applications; future prospects; bio-potentiality

1. Introduction

Microbial metabolites present a myriad of opportunities for diverse future prospects. Certain microorganisms, such as fungi and mushrooms, exhibit notable potential in the realms of pharmacology and medicine. Their inherent properties hold promise for various medicinal applications. These organisms contribute significantly to the development of pharmaceutical and therapeutic advancements. The use of microbes in therapeutic research is a fundamental component of the hunt for new pharmaceuticals, especially when it comes to investigating natural compounds. Because of their inherent qualities, fungi and mushrooms stand out as excellent examples with enormous promise in the fields of pharmacology and medicine. Endophytic fungi are of special interest since they are found inside a variety of plants and are the focus of research projects. These fungi have symbiotic connections with their host plants, which greatly enhance the resilience and health of the plants. There is great pharmacological potential in the wide variety of metabolites they produce, which include phenolic chemicals, alkaloids, flavonoids, steroids, and terpenoids[1]. These metabolites frequently display an amazing range of properties, such as antibacterial, anticancer, antioxidant, and anti-diabetic benefits. Endophytic fungi offer themselves as excellent resources for natural medical drug discovery by enhancing plant resilience and producing compounds with therapeutic relevance. Research on the diverse characteristics of plants is still ongoing. These include their ability to act as sources of firewood, hardwood, gum, tannins, and feed, as well as their symbiotic interactions with these fungi. Plants and their endophytic fungi have a symbiotic relationship that provides a rich environment for the discovery of novel bioactive chemicals with medicinal promise. All things considered, microbial metabolites particularly those derived from endophytic fungi found inside plants—offer exciting opportunities for the creation of novel therapies that are both environmentally friendly and highly effective[2].

Pharmacology and medicine are profoundly impacted by endophytic fungi, which are important contributors to the synthesis of bioactive substances with antibacterial qualities. These fungi are found living inside different kinds of plants and form symbiotic connections with them without harming the host plants. Rather, they have favorable effects on the physiological processes of the host plant, enhancing its general robustness and health. The production of bioactive compounds, such as those with antibacterial qualities, is one important contribution made by endophytic fungus. These substances are classified into a wide range of chemical classes, including steroids, terpenoids, alkaloids, flavonoids, and phenolic chemicals. Each of these compounds has unique pharmacological properties, most notably antibacterial actions[3]. Their structural distinctiveness and adaptability make them important tools for the development of natural therapeutic drugs. Because they produce secondary metabolites, endophytic fungi have a dual function of protecting plants from microbiological dangers and enhancing their health. These metabolites have therapeutic value in addition to bolstering the plant's defenses against biotic and abiotic stresses. These fungi broaden the range of phytochemical applications in medicine by aiding in the synthesis of phytochemicals having medicinal potential. Discoveries concerning the function of endophytic fungi in synthesising antimicrobial compounds are what propel advances in the field of natural product discovery. These investigations not only broaden our knowledge of microbial metabolites but also open new avenues for the creation of novel therapies that are more effective and have less negative effects on the environment. There is promising research as studies into the complex link between plants and their endophytic fungi continue[4].

The goal of this review paper is to provide a thorough analysis of the discovery of antimicrobial chemicals found in endophytic fungi that live in *Anogeissus latifolia*, an uncommon but useful plant found in central India. The goal of the research is to progress the discovery of natural products for medicinal uses by concentrating on this particular species. It aims to provide comprehensive pharmacological understanding of these substances, illuminating their modes of action. Furthermore, the paper explores the symbiotic interaction between the plant and its fungi, clarifying how they contribute to defensive systems and the synthesis of bioactive substances. The review article aims to support ecologically friendly techniques by highlighting ecological aspects and promoting sustainable healthcare practices. In essence, its goal is to enhance comprehension of the bioactive capabilities of endophytic fungi, which will enable the creation of novel therapeutic interventions. Small to medium-sized trees endemic to South Asia and Africa are called axlewood, or *Anogeissus latifolia*. Its large, straight, cylindrical leaves with simple, grayish-yellow or whitish hairs beneath them are its defining features. It is a member of the Combretaceaefamily[5]. Fruits with two wings, called pseudo-achenes, are produced by the tree. A notable source of bioactive chemicals, especially antibacterial agents, *Anogeissus latifolia* is one of the therapeutic plants that warrant investigation. Scientists have started a bioprospecting expedition in this tree to find the antimicrobial compounds that endophytic fungi living inside of it secrete. These bioactive compounds are identified and described in this ground-breaking work using advanced analytical approaches such as metabolomics. Beyond its therapeutic uses, *Anogeissus latifolia* is significant. The tree provides a variety of resources, including gum, tannins, heavy hardwood, great firewood, and feed for cattle and buffaloes. It is a major fuel, fodder, and lumber tree in many areas, especially the foothills of the Indian Himalaya. On the other hand, unsustainable activities like overclipping leaves and felling firewood could result in insufficient regrowth of *Anogeissus latifolia* populations. For this reason, maintaining the ecological and social benefits of this tree species depends on its preservation and sustainable use. The investigation into *Anogeissus latifolia* as a possible source of antibacterial compounds emphasizes how crucial it is to preserve biodiversity and use natural resources sustainably. Researchers hope to advance medicine by comprehending and utilizing the therapeutic qualities of plants like *Anogeissuslatifolia*[6].

In order to realize *Anogeissuslatifolia*'s potential as a medicine, the research is based on a thorough investigation of the plant's chemical components and pharmacological characteristics. By means of thorough chemical investigation, important substances including 4,'-β-D-glucoside, 3,3'-di-O-methyl, and 3,4,3'-tri-O-methylflavellagic acid 4'-β-D-Xyloside have been identified, demonstrating their possible medical uses. Pharmacological studies have shed further light on the many traditional applications of *Anogeissus latifolia*, which include soothing skin disorders, curing fever and cough, and relieving scorpion stings. To further illuminate the plant's complex medicinal effects, the study explores its diuretic and wound-healing capabilities[7]. Interestingly, thorough evaluation of *Anogeissuslatifolia*'s antibacterial properties has shown encouraging results against a range of diseases. By carefully examining bioactive components, the study highlights how important *Anogeissus latifolia* is for drug development efforts. These endeavors not only highlight the significance of *Anogeissus latifolia* as an invaluable asset in the field of herbal medicine, but they also open the door for the creation of innovative therapeutic approaches. The work adds to our understanding of this botanical species' medicinal potential by elucidating its complex chemistry and pharmacological characteristics, underscoring its importance in contemporary healthcare practices[8].

The thorough investigation of *Anogeissuslatifolia*'s medicinal potential combines several approaches, including microbiological screenings, pharmacological testing, and chemical analysis. The careful chemical analysis identified important compounds, and the use of cutting-edge methods like High-Performance Thin Chromatography (HPTLC) accelerated the quantification of important flavonoids, such as quercetin and rutin, simplifying the identification of active chemicals. Furthermore, pharmacological analyses clarified the plant's traditional therapeutic uses, confirming its efficacy in treating a variety of illnesses such as fever, cough, and skin disorders. Additionally, the evaluation of its antibacterial, diuretic, and wound-healing qualities produced encouraging outcomes against a wide range of infections[9]. The discovery from antimicrobial screening that certain extracts have strong inhibitory effects on the growth of microorganisms is noteworthy and highlights the potential therapeutic use of these extracts. Additionally, the study broadened its focus to include the bioprospecting of microbial endophytes found in *Anogeissus latifolia*. By utilizing morphological traits and genomic DNA analysis, new bioactive metabolites were identified, providing a favorable environment for the development of ground-breaking medications. Finally, bioactive compounds were carefully characterized for their antimicrobial potential using rigorous screening techniques like the Agar well diffusion assay and micro-broth dilution assay. This provided priceless insights into the plant's medicinal qualities and laid the groundwork for the development of novel antimicrobial agents[10].

The research findings have consequences for several fields and show great promise for therapeutic breakthroughs. The study provides insights into the wide therapeutic potential of *Anogeissus latifolia* by identifying important chemical elements and pharmacological features. The plant may be useful in treating a variety of illnesses, from fever and cough to skin disorders and microbial infections, according to the discovery of bioactive components and their antibacterial qualities[11]. Furthermore, investigating endophytic fungus within the plant offers chances to find new bioactive compounds, opening the door for the creation of cutting-edge medications. All things considered, these results highlight the significance of *Anogeissus latifolia* in herbal medicine and drug discovery, potentially making a big impact on therapeutic advancements[12].

2. Chemical constituents

This systematic review comprehensively analyzed the impact of chemical constituents and its pharmacological activities of bark, leaves, stem and Seeds of *Anogeissus latifolia*, particularly focusing on its anti-diseases tendency like kidney failure, nephroprotective effects etc. Previous studies have identified several bioactive compounds in A. latifolia. Notably, 3,3-di-O-methyle ellagic acid 4'-β-D-Xyloside, 3,4,3'-tri- O-methyl flavellagic acid 4'-β-D-glucoside, and a steroid, 3-β-hydroxy-28-acetytaraxaren, were isolated from the ethyl acetate fractions of the bark. Additionally, a method employing High-Performance Thin Chromatography (HPTLC) was developed for the quantification of rutin and quercetin, revealing rutin content of 0.1617% w/w and quercetin content of 1.875% w/w in the hydroalcohol bark extract[13]. The ethanolic extract of A. latifolia demonstrated nephroprotective potential in a rat model of gentamicin-induced nephrotoxicity. This effect was attributed to the presence of antioxidant and anti-inflammatory phytoconstituents, including ellagic acid. Moreover, qualitative analysis of methanol extracts and fractions revealed the presence of flavonoids, carbohydrates, phenols, alkaloids, saponins, and glycosides[14]. Further, high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis identified twenty-two bioactive ingredients, mainly flavanones, contributing to the pharmacological profile of A. latifolia. While the quantification method using HPTLC provides valuable data on rutin and quercetin content, it is essential to acknowledge potential limitations. The focus on these two compounds may overlook other bioactive constituents with pharmacological significance[15].

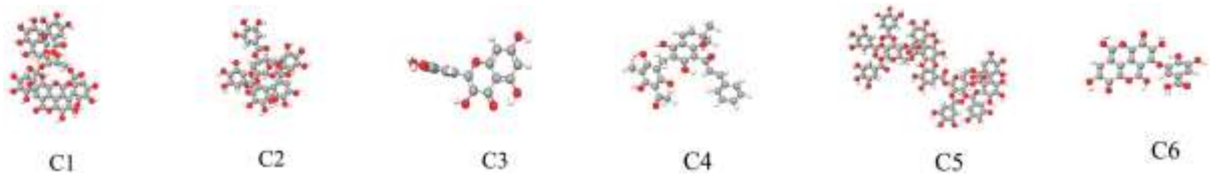

Figure 1. (*Continued*).

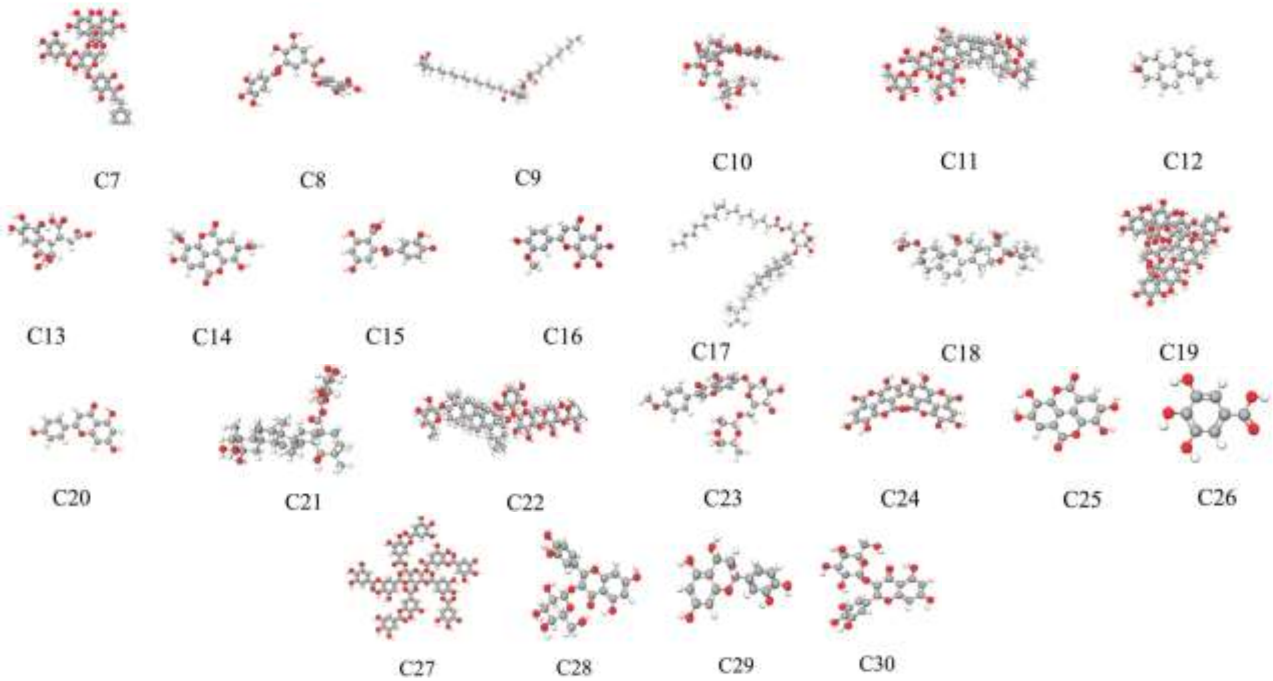

Figure 1. Majorly active therapeutic chemical constituents: C1: Punicacortein D, C2: Punicafolin, C3: Quercetin, C4: Rottlerin, C5: Rugosin D, C6: Sanguisorbic acid dilactone, C7: Thonningianin A, C8: Trigallic acid, C9: Uvaricin, C10: Rutin, C11: Saponins, C12: Sterol, C13: Chebulic acid, C14: 2,8-Di-O-methylellagic acid, C15: 2-Protocatechoylphloroglucinolcarboxylate, C16: 4',5,6,7,8-Pentahydroxy-3'-methoxyflavone, C17: 18 1-Glc-Sitosterol, C18: 22-epi-Hippurin-1, C19: Acutissimin A, C20: Apigenin, C21: Arjunglucoside I, C22: Asterlingulatoside D, C23: Biochanin A 7-O-rutinoside, C24: Diellagilactone, C25: Ellagic acid, C26: Gallic acid, C27: Gallotannin, C28: Isoquercitrin, C29: Leucocyanidin, C30: Myricetin 3-galactoside.

Anogeissus latifolia Roxb., contain myriad of bioactive compounds with therapeutic significance[16,17]. These compounds include Thonningianin A, 3-di-O-methylflavellagic acid-4'-β-D-xyloside, Gallotannin, 3,3',4-tri-O-methyl ellagic acid, Punicacortein D, 3-di-O-methylflavellagic acid, 22-epi-Hippurin-1, 3,3'-di-O-methyl ellagic acid-4'-β-D-glucoside, 25-Acetyl-6,7-didehydrofevicordinF3-glucoside, 19-Hydroxydeacetylnomilinic acid 17-betaD-glucopyranoside, Arjunglucoside I, Luteolin-7-O-β-D-galactopyranoside, Rottlerin, Acutissimin A, Quercetin, Myricetin, Luteolin, Quercetin-3-O-β-D-galactopyranoside, 4,3'-tri-O-methyl flavellagic acid-4'-β-D-glucoside, Sanguisorbic acid dilactone, 18:1-Glc-Sitosterol, Isoquercitrin, Biochanin A 7-O-rutinoside, Rugosin D, Uvaricin, 2,8-Di-O-methylellagic acid, 1,2'-Di-O-galloylhamamelofuranose, Cyanidin 3-(6-feruloyl-2'-sinapoylsophoroside) 5-glucoside, 2-Protocatechoylphloroglucinolcarboxylate, Punicafolin, and Asterlingulatoside D. Moreover, the presence of saponins and sterols further adds to the pharmacological importance of *Anogeissuslatifolia*[18,19]. These plethoras have distinct pharmaceutical significance that shows multifaceted results towards the pharmaceutical industries and health sectors. Thonningianin A shows promise in combating cancer and exhibits antioxidant properties. 3-di-O-methylflavellagic acid-4'-β-D-xyloside contributes antioxidant and anti-inflammatory effects. Gallotannin displays antioxidant, antimicrobial, and anti-inflammatory activities. Meanwhile, 3,3',4-tri-O-methyl ellagic acid demonstrates antioxidant and anti-inflammatory effects, potentially aiding in cancer prevention. Punicacortein D presents antioxidant and anti-inflammatory properties, alongside potential anticancer effects. Other notable compounds like 3-di-O-methylflavellagic acid, 22-epi-Hippurin-1, and 3,3'-di-O-methyl ellagic acid-4'-β-D-glucoside exhibit antioxidant, antimicrobial, and antifungal properties[16,17]. Additionally, 25-Acetyl-6,7-didehydrofevicordinF3-glucoside displays antioxidant properties and potential anticancer effects. Compounds such as 19-Hydroxydeacetylnomilinic acid 17-betaD-glucopyranoside demonstrate potential neuroprotective and antioxidant effects. Arjunglucoside-I exhibit antioxidant and hepatoprotective effects, while Luteolin-7-O-β-D-galactopyranoside contributes to antioxidant and anti-inflammatory activities[18,19]. Rottlerin shows antioxidant properties and potential anticancer effects, and Acutissimin A displays antioxidant and anti-inflammatory effects. Furthermore, Quercetin, Myricetin, and Luteolin exhibit a wide array of pharmacological actions including antioxidant, anti-inflammatory, anticancer, and neuroprotective effects[20]. The Boll-Stick Structure of therapeutic bioactive compound is depicted in **Figure 1**.

3. Pharmacological and antimicrobial properties

The bark and leaves are useful for tanning, and the wood is significant. Bark is useful for piles and anemic urine discharges. Stem bark has astringent, hemostatic, constipating, depurative, and kapha and vata vitiated properties. Jain claims that it helps with skin conditions, snakebite, colic, cough, diarrhea, dysuria, and liver problems. This tree's bark is used by the tribes in the Rajasthani area of Udaipur to treat fever. Bark is known as "DangyaKhokala", a cure for persistent cough. Tribal people who live in the Gundlabranhmeswaram Wild Life Sanctuary's forest employ stem bark paste to scorpion stings[21]. Bark infusion-two spoons twice a day-relieves coughing, while leaf infusion-which is typically used after delivery-works well as a tonic for epileptic episodes. These chemicals were densitometrically determined in the reflection/absorptive mode at 366 nm. *Anogeissus latifolia* was discovered to contain 1.875%w/w of quercetin and 0.1617%w/w of rutin, respectively. With rutin and quercetin having $r = 0.9997$ and $r = 0.9942$, respectively, the calibration plots demonstrated a strong linear connection. Rutin and quercetin showed remarkable repeatability, with an average recovery of 99.98% and 100.11%, respectively. The method's reproducibility was demonstrated by data analysis[22]. It was shown that the HPTLC approach was easy to use and practical for quickly screening active compounds and quantifying the studied flavonoids in the bark and leaves are utilized for tanning, and the timber is significant. Bark works well in anemic environments. Astringent, constipating, depurative, and helpful in vitiated conditions is stem bark. Stem bark is beneficial for diarrhea, dysuria, coughs, colic, liver problems, snakebite, and skin conditions[12]. The bark of this tree is used by the tribal people of Rajasthan's Udaipur district for medicinal purposes. "Dangya Tribal people residing in the forest of Gundlabranhmeswaram Wild Life Sanctuary apply paste of bark as a remedy for chronic cough." Bark decoction can be taken twice a day to treat coughs, while leaf decoction can help with epileptic seizures. Gum is typically taken as a tonic following delivery[23].

Using an in-vivo Lipschitz test technique, the diuretic ability of methanol and aqueous extracts of the leaf sections was evaluated in albino rats. The study's parameters were urine volumes and potassium and sodium ion concentrations in the urine. The conventional treatment was furosemide[18]. The findings reveal that when compared to the control, there is a substantial ($p < 0.05$) increase in urine volume and electrolyte excretion ($p < 0.05$) with methanol and aqueous extract at 500 mg/kg body weight. There is noticeable diuretic action in both extracts. The current investigation led to the conclusion that the compounds found in methanol and aqueous extracts might be in charge of the diuretic effect. By reducing the wound's surface area and strengthening its tensile strength, *Anogeissus latifolia* quickens the healing process. The positive control in this experiment was nitrofurazone ointment. 15 days were needed to observe complete epithelization with *Anogeissus latifolia*. The hydroxyproline level and the healed area's measurements agreed[24]. β-sitosterol and 3-β-hydroxy-28-acetyltaraxaren were extracted using ethyl acetate from the stem bark of *Anogeissus latifolia*. After being put through an antimicrobial screening process, the ethyl acetate and methanol extracts shown a strong inhibitory effect on microbial growth[25].

Antioxidant capability of *A. latifolia* assessed with 50% of ethanolic extract and found that the free radicals nitric oxide, DPPH, hydrogen peroxide, and superoxide were all inhibited at different doses. *Anogeissuslatifolia*'s capacity to lower serum cholesterol levels in albino rats is hypolipidemic[26]. In hyperlipidemic-induced rats, they observed that gum ghaati therapy dramatically decreased the levels of total cholesterol and triglycerides at 500 mg and 750 mg/kg of body weight; also, the high density lipoprotein cholesterol was enhanced at the 750 mg/kg of body weight dose[27]. In vitro: *Anogeissus latifolia* extract and CCl4 were applied to primary hepatocyte monolayer cells. The primary monolayer culture injured by CCl4 showed signs of protective action. In vivo: Serum marker enzyme activity indicated that *Anogeissus latifolia* hydroalcoholic extract (300 mg/kg) protected rats' livers from CCl4-induced damage[25]. There was a description of the antibacterial properties of aromatic and medicinal plants from different nations, along with a summary of the results. In a different investigation, plant extracts from *Tagetes patula*, *Achyranthes aspera*, and *Lantana camara* were shown to have antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*[28].

4. Bioprospecting of microbial endophytes for novel biologically active metabolites

The discovery of new medications is still greatly aided by natural products. The identification of new compounds with potential use as medicines can be facilitated by the study of microbial natural products. The hunt for biologically active chemicals has frequently been prompted by the emergence of novel developing diseases and medication resistance among pathogenic strains. Therefore, it is imperative to explore microbial sources for innovative pharmaceuticals for use as therapeutic agents[29]. Because they produce secondary metabolites, which could one day be used as pharmaceuticals, microorganisms have made enormous contributions. The endophytic fungus Taxomycesandreanae has successfully produced the anticancer medication Taxol, which has opened up new possibilities for the bioprospecting of microbial endophytes for novel bioactive compounds. Considering this idea, certain endophytes generate bioactive compounds that resemble the unique metabolites of host plants[12]. The precious wood *Anogeissus latifolia* is used for tanning its leaves and bark. It works well for treating piles, diarrhea, dysuria, cough, colic, liver problems, snakebite, and skin conditions. The bark is used by Rajasthani tribes to treat fever and a persistent cough known as "DangyaKhokala". In the Gundlabranhmeswaram wild life sanctuary, tribal people bandage scorpion bites with a stem bark paste. Bark decoction is used as a daily cough treatment, and leaf decoction helps with epileptic seizures. After delivery, gum is eaten as a tonic. Rutin concentration in *Anogeissus latifolia* was found to be 1.875% w/w and 0.1617% w/w by densitometric measurement of quercetin. For the quick screening of active compounds and measurement of studied flavonoids, the HPTLC approach proved to be easy to use and practical. In Fuyang County, Zhejiang Province, China, plant material from mature *A. macrosperma* was collected between August 2009 and November 2009[30]. Parts that were clear of disease were removed and placed in sterile plastic bags. Using a modified technique, endophytes were separated by rinsing the material in sterile demineralized water after it had been surface-disinfected with 70% ethanol and 0.1% mercuric chloride and cleaned in sterile water. The sample was subsequently spread out onto aqueous agar supplemented with streptomycin, and it was kept there for 28 ± 2 °C until fungal growth started. On mycological media, such as potato dextrose agar or Sabouraud agar, the tips of the fungal hyphae were positioned. Colony morphology was used to evaluate each fungal culture's purity. The last pure cultures were put into PDA slant tubes, and to look for contaminated fungus, uncut, surface-disinfected, and nondisinfected pieces were put on the same agar[31]. The study focuses on the identification of endophytic fungi based on morphological characteristics, including colony descriptions, growth rates, and microscopic observations. The fungi were then analyzed for genomic DNA extraction using the Qiagen DNessay Mini Kit, electrophoresis, purification, and sequencing. The sequences were manually edited and compared with available data from GenBank databases using the BLASTNprogram[32]. Another study reported that the genomic DNA of the isolate was isolated and amplified and sequenced using universal primers ITS4 and ITS5. The contig obtained from assembling forward and reverse reads were deposited to GenBank with the accession MN128230.1. A BLAST homology searches ITS rDNA against the NCBI non-redundant nucleotide database showed the isolate resembling *Colletotrichum coccodes*. A total of 995 ITS rDNA sequences belonging to different *Colletotrichum spp*. were randomly retrieved from GenBank and screened for the presence of complete ITS rDNA region[30]. Phylogenetic analysis was performed using selected 83 sequences, and the tree showed clustering of the isolate under the clade C. coccodes. The MP phylogenetic tree displayed the isolate's close affinity toward *C. coccodes*, supported by a bootstrap value[33].

5. Bioactive compound production and screening for antimicrobial potentiality

In the realm of bioactive compound production and screening for antimicrobial potentiality, researchers meticulously investigate natural sources to isolate substances with therapeutic properties. This process involves rigorous screening methodologies to identify compounds that exhibit antimicrobial efficacy, paving the way for the development of novel drugs to combat infectious diseases. A BOD shaking incubator was used to cultivate the fungus and incubate it for two weeks while the broth was made from potatoes and water. Following filtration, ethyl acetate (EtOAc) was used to extract fungal metabolites by vigorously shaking the soup. Analyzing the organic extract's antibacterial efficacy against therapeutically relevant test pathogens by an Agar well diffusion assay, the extract's weight was identified. Together with one pathogenic fungus, *Candida albicans*, the test organisms comprised three bacterial pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*[34]. Nutrient agar (NA) and Sabouraud dextrose agar (SDA) medium were used to cultivate the test organisms in order to activate them. For every plate, a grass culture was created, and 100 µl of EtOAc organic extracts diluted in 10% dimethyl sulfoxide (DMSO) were added to agar wells, growing the test organisms on nutrient agar (NA) and sabouraud dextrose agar (SDA) media, the organisms were rendered active. Each plate was prepared with a grass culture, and 100 µl of EtOAc organic extracts diluted in 10% dimethyl sulfoxide (DMSO) were added to agar cups. The study aimed to determine the minimum inhibitory concentration (MIC) of an organic extract of *C. coccodes*, an endophytic plant, against *P. aeruginosa*, *S. aureus*, *E. coli*, and *C. albicans* using a micro-broth dilution assay in a sterile 96-well plate. The extracts were diluted with different concentrations, and the MIC was determined as the lowest concentration of the test extract at which no pink color appeared. It showed that the extract exhibited considerable antimicrobial activity against all tested pathogens, with maximum inhibition observed against *S. aureus* followed by *E. coli* and *C. albicans*. The MIC for *S. aureus*, *E. coli*, and *C. albicans* was found to be 125 µg/ml, while that for *P. aeruginosa* was 250 µg/ml. The bioactive extract of *C. coccodes* was characterized by its metabolite tyrosol, which has a strong affinity toward bacterial tyrosyl tRNA synthetase and fungal CYP45014α-lanosterol demethylase, suggesting its possible antimicrobial action mechanism[35]. The isolate was characterized by it's ITS rDNA and ITS2 sequence, and the ITS2 structure was used to differentiate the isolate from the same species of different lifestyles.

The findings could be useful in exploring the bioactive potential of fungal endophytes colonizing plants of ethnomedicinal importance and potentially as a marker for species differentiation among dissimilar lifestyles[36]. The antibacterial and antifungal properties of several solvent extracts, including Cassia fistula extract, are the main focus of the investigation. The antibacterial and antifungal properties of these extracts were screened using the agar well diffusion method. Molten cooled Muller Hinton agar (MHA) or Potato dextrose agar (PDA) was added to the inoculums after fresh bacterial or fungal cultures were pipetted into sterile Petri dishes[37]. Pre-experiments and earlier research were used to determine the extract concentration (20% w/v). To find the extracts' minimum inhibitory concentrations (MIC), their potency against food pathogens and spoilage bacteria was adjusted by varying their concentration. After preparing various extract concentrations using a two-fold serial dilution method, the plates were incubated for eighteen hours at 37 °C. It was thought that the MIC. The MIC was considered the lowest concentration that inhibited the growth of the respective microorganisms[35]. Hydroalcohol extracts' in vitro antibacterial and antifungal properties were investigated. Using the agar disk diffusion method, the antibacterial and antifungal properties of plant component extracts were examined against four pathogenic bacteria and three pathogenic fungi. The agar cup method was utilized to assess the antibacterial activity, and standard antibiotics in the form of pure strains of Gram-positive, Gram-negative, and fungal bacteria were taken for comparison[38]. The antibacterial and antifungal properties of all the extracts were tested against the bacteria *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes,* and the fungus *Aspergillus niger, Aspergillus clavatus,* and *Candida albicans*. The zone of bacterial growth inhibition was used to evaluate the antibacterial and antifungal properties of the extracts. The findings demonstrated that the extracts' antibacterial and antifungal properties rose linearly as extract concentration increased. For all susceptible bacterial strains, the measured growth inhibition zone varied from 11 to 20 mm, while for fungal strains, it was between 14 and 20 mm[39]. Using potato dextrose agar medium (PDA) and a temperature of 25 °C, Seven pathogenic fungi, including *Candida albicans, Rhizoctonia solani, Sclerotinia sclerotiorum, Botrytis cinerea, Fusarium graminearum, Magnaporthe oryzae,* and *Phytophthora capsici*, were cultivated for three to six days to generate fresh mycelia for subsequent antifungal experiments. The mycelium growth inhibition method was employed to evaluate the antifungal activity of *P. harmala*. Outcomes of *P. harmala* extract's antifungal effectiveness against pathogenic fungi[40]. The methanol extract of seeds efficiently inhibited seven fungi: *Phytophthora capsici*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium graminearum*, *Magnaporthe oryzae*, and *Candida albicans* ATCC 24433, with inhibition rates of 50.33%, 64.32%, 76.32%, 52.98%, 42.57%, 55.58%, and 47.47%, respectively. The concentration of the extract of seeds was 500 μgmL^{-1}. *Rhizoctonia solani* and *Magnaporthe oryzae* were moderately inhibited by the petroleum ether extract of seeds, with inhibition rates of 10.88%. *Magnaporthe oryzae* and *Fusarium graminearum* were only marginally inhibited by the methanol and partial petroleum extract of aerial portions, with inhibition rates of 10.89% and 10.7% and 12.08% and 16.57%, respectively. With a broad antifungal range, the methanol extracts of seeds exhibited more potent antifungal activity. Our findings demonstrated the broad-spectrum antibacterial activity of the aerial parts and seed extracts against agricultural pathogenic bacteria, which may be related to their alkaloids[39,41]. However, more research is required to determine the precise active ingredients and the antibacterial mechanism of these extracts. The objective of the study was to examine the levels of vasicine, vasicinone, harmine, and harmaline in *P. harmala* seeds and aerial parts. Methanol was used to create standard chemical solutions with varying gradient concentrations. The samples underwent a 30-minute, 30 °C ultrasonic treatment with methanol to extract them, after which they were filtered for HPLC analysis. EasySep-3030 liquid chromatography, including an EasySep-

3030 UV detector, AS-2000 automatic sampler, and EasySep-3030 binary pump, was utilized for the HPLC analysis. Vasicine, vasicinone, harmaline, and harmine were shown to have relative retention time[40]. The chromatographic conditions specified in the HPLC analysis section were followed in the preparation of the sample solution. Calculations were made for the amounts of vasicine, vasicinone, harmaline, and harmine in seeds as well as the amounts of vasicine and vasicinone in aerial portions. No harmine or harmaline was found in the aerial sections. The study offers insightful information about the characteristics and composition of P. harmala powders. Another study reported towards pharmacological studies for entomopathogenic medicinal mushroom *Cordyceps* species that has been contributing to biological and pharmacological health benefits for years, containing diverse bio-metabolites with extensive activities[42]. One key constituent, cordycepin, plays a crucial role in numerous biochemical and molecular processes. Despite ongoing interest, challenges persist in terms of excessive harvesting, commercial cultivation, and the infrequent formation of artificial stromata. The genus *Cordyceps* exhibits genomic expansion, and there is a significant demand for studies on mating-type genes identification, metabolite-producing genes, and strain improvement techniques[43,44].

6. Conclusion

In conclusion, the discovery of novel, safe, effective, and low-toxic medications derived from natural sources is the emphasis of pharmaceutical chemists these days. Because the amount of new compounds to be extracted in the future tends to expand exponentially and fast, endophytic fungi may serve as renewable sources of novel bioactive compounds with pharmacological properties. Moreover, a number of investigations have revealed that the bioactive substances that were separated from the endophytic fungus are also found in plants and exhibit comparable biological properties to those found in plant sources. Thus, we draw the conclusion that endophytic fungi could be the most advantageous substitute for utilizing pharmacological bioactive substances in the creation of medications intended for both human and animal use. Therefore, new endophytic fungal compounds with pharmacological activity must be found, and their mechanisms of action must be clarified using pre-clinical, biochemical, pharmacodynamic, biological, and bioinformatics techniques. One of the most significant medicinal plants used in Ayurvedic medicine for heart disorders is *Anogeissus latifolia*. The herb helps with fever, epileptic episodes, skin conditions, liver problems, UTI infections, and more. Ellagic acid, a phenolic phytoconstituent that is pharmacologically active, is abundant in the plant. It has the ability to cure, has microbicidal activity, is antiulcer, hypolipidemic, and hepatoprotective properties. The current review summarizes research on this therapeutic plant from the fields of ethnobotany, phytochemistry, pharmacology, and biotechnology.

7. Hypothesis

The hypothesis of this study is that endophytic fungi from *Anogeissus latifolia* contain bio-metabolites that have high antibacterial potential but have not yet been discovered. Endophytic fungus will become increasingly attractive as sources of novel bioactive chemicals as the pharmaceutical industry accelerates its efforts to find safe and effective drugs derived from natural sources. Previous research will show that these chemicals have pharmacological effects similar to those found in plants. The proposal posits that investigating the pharmacological properties of novel endophytic fungal compounds derived from *Anogeissus latifolia* will reveal bio-metabolites possessing a variety of antibacterial characteristics. In order to identify the mechanisms of action, the study will use a thorough strategy that includes pre-clinical, biochemical, pharmacodynamic, biological, and bioinformatics tools.

Furthermore, investigating *Anogeissus latifolia*, a medicinal plant abundant in the bioactive substance ellagic acid, would support the search for alternative therapies. The plant will be positioned as a valuable source for pharmaceutical research due to its traditional use in Ayurvedic medicine for different diseases, including cardiac issues. The proposal posits that by clarifying the antibacterial capabilities of endophytic fungus originating from *Anogeissus latifolia*, new substances with potential applications in veterinary and human medicine will be found. The goal of the research is to uncover the antimicrobial potential of endophytic fungi, discover the "yureka" in bio-metabolites, and advance our knowledge of pharmacologically active natural chemicals.

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Conflict of interest

The authors declare no conflict of interest.

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