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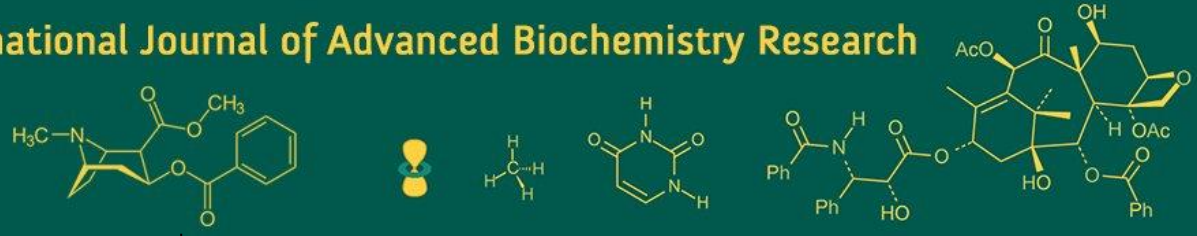

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Endophythic fungi in bamboo (*Dendrocalamus giganteus*): Analysis for phytochemicals and antimicrobial property

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Abstract
This study investigates the presence and diversity of endophytic fungi in the bamboo species *Dendrocalamus giganteus*, focusing on their phytochemical profiles and antimicrobial properties. Endophytes, which inhabit plant tissues symbiotically, have gained attention for their potential to produce a wide array of bioactive compounds. Samples were meticulously collected from various parts of the bamboo, including leaves, stems, and roots, then isolated and cultured under controlled conditions to identify and characterize the fungal species present. Through phytochemical analysis, it was determined that the fungal extracts contained significant amounts of terpenoids, alkaloids, flavonoids, and carbohydrates, compounds known for their therapeutic potentials.

These findings advocate for continued exploration and characterization of bamboo endophytes, which could lead to significant advancements in natural product-based drug discovery and development.

Keywords: Endophyt, fungi, bamboos, *Dendrocalamus giganteus*, phytochemical, antimicrobial

Introduction

The term "endophyte" originates from the Greek words "endon" (meaning within) and "phyton" (meaning plant), signifying an organism that lives within a host plant. This term is widely used across different organisms and hosts, including bacteria (Kobayashi and Palumbo 2000) [1], fungi (Stone *et al.*, 2000) [2], plants (Marler *et al.* 1999) [3], insects within plants (Feller *et al.*, 1995) [4], and algae within algae (Peters *et al.*, 1991) [5]. Endophytes can colonize any part of the host plant, and their relationships with hosts vary widely from facultative saprobic to parasitic to mutualistic.

The term "endophyte" encompasses diverse symbiotic strategies, such as pathogenic endophytic algae (Bouarab *et al.*, 1999) [6], parasitic endophytic plants (Marler *et al.* 1999) [3], and mutualistic endophytic bacteria and fungi (Chanway 1996; Adhikari *et al.*, 2001; Bai *et al.* 2002) [7, 8]. It also includes pathogenic bacteria and fungi in latent phases (Sinclair and Cerkauskas 1996) [8], along with microorganisms in communalistic relationships (Sturz and Nowak 2000) [9].

The definition of "endophyte" often refers to organisms whose infections are inconspicuous, causing no apparent symptoms in the host tissues, and can be internally demonstrated (Stone *et al.*, 2000) [2]. This definition encompasses a range of microorganisms with different life strategies, including those that grow saprophytically on dead tissues after an endophytic phase, avirulent microorganisms, latent pathogens, and virulent pathogens in early infection stages.

These symbiotic relationships between the endophytes and host plants have been found to enhance plant growth, increase resistance to biotic and abiotic stress, and even contribute to the production of active bioactive compounds with potential agricultural and pharmaceutical applications (Altemimi *et al.*, 2017).

These host creates perfect ecosystem and reservoir for microorganisms to dwell (Morakotkarn *et al.*, 2007) the study of endophytes has opened up new avenues for understanding complex interactions between plants and microorganisms, highlighting the importance of these hidden allies in shaping the health and productivity of plant ecosystems.

Fungi

Fungi are an incredibly diverse group that are essential to ecosystem functioning. They play a key role in recycling energy and nutrients and influence plant community composition through symbiotic relationships (Dighton *et. al.*, 2003). Fungal fruiting bodies (sporocarps) are consumed by a variety of invertebrate and vertebrate animals, including humans (Arora, 2001; Johnson, 1996; Lilleskov & Bruns, 2005). Despite their recognized importance, our knowledge about fungal diversity and ecology remains limited. For example, although an estimated 1.5 million species of fungi are believed to exist, only 72,000 have been described (Hawksworth *et. al.*, 2005). Recently, there has been concern about the decline of many fungal species worldwide due to human activity (Arnolds *et. al.*, 1991; Watling, 2005). Therefore, more research is needed to understand fungal diversity, their ecological roles, and the impact of human activities on fungal diversity and function.

Fungal diversity can be categorized either taxonomically or by their functional roles. The latest classification schemes identify six phyla and four unplaced subphyla (Hibbett *et al.*, 2007; James *et al.*, 2006a; James *et al.*, 2006b), comprising five major groups: Chytridiomycota, Zygomycota, Ascomycota, Basidiomycota, and Glomeromycotan. Notably, the functional roles of fungi frequently span across these taxonomic boundaries.

Bamboo: Bamboos, belonging to the grass family Poaceae, are renowned for their rapid growth, structural versatility, and ecological significance. These perennial plants are widely distributed across diverse habitats, ranging from tropical forests to temperate regions, and play essential roles in ecosystem functioning and human livelihoods (Li *et al.*, 2016) [10]. Bamboos are characterized by their woody stems, known as culms, which exhibit exceptional strength, flexibility, and resilience.

Endophytic fungi have been documented in various species of bamboo, where they inhabit different plant tissues, including roots, stems, and leaves. Studies have revealed a high diversity of fungal endophytes associated with bamboo species, with representatives from both Ascomycota and Basidiomycota phyla (Huang *et al.*, 2019) [11]. These endophytes contribute to the health and resilience of bamboo forests by enhancing nutrient uptake, improving stress tolerance, and protecting against pathogens and herbivores.

Furthermore, endophytic fungi in bamboos have been found to produce bioactive compounds with potential applications in medicine, agriculture, and industry. For instance, extracts from endophytic fungi isolated from bamboo have shown antimicrobial and antioxidant activities, suggesting their potential for pharmaceutical and nutraceutical purposes (Wang *et al.*, 2019) [12]. The exploration of endophytic fungi in bamboos represents an emerging area of research with implications for both ecological understanding and biotechnological innovation.

Dendrocalamus giganteus

Dendrocalamus giganteus belongs to the family Poaceae, is one of the largest bamboo species. D. *giganteus* is one of the twelve high yielding bamboos worth raising as a large scale bamboo plantation, as it is very good for construction, paper production and young shoots are good for 2 vegetable products. Culms are also used for scaffolding, boat masts, rural housing, water pipes, vases, buckets, water pitchers, matting, boards and parquet, furniture, water pots. Culm sheaths are used to make hats. The stems are especially suitable for the production of bamboo boards, which is an ideal material for room decoration and other interior applications such as walls, ceilings, floors, doors, shelves, etc. *D. giganteus* can be planted to protect soil against erosion. As one of the largest bamboo species, it has a high ornamental value. This bamboo species also produces large amount of biomass. It can give an annual yield of 20 to 30 t/ha, which is 2.7 times higher than that of *D. latiflorus* (Schröder *at al.*, 2010).

Materials and Methods

Phytochemicals screening: Phytochemical screening refers to the extraction, screening, and identification of the medicinally active substances found in plants (Harborne *et al.*, 1995) [13]. Phytochemicals are naturally occurring bioactive compounds found in the leaves, stems, bark, roots, fruits, and seeds of plants that serve as a defense mechanism and protect against various diseases (Dewick *et al.*, 2009) [14]. Phytochemicals are categorized into primary and secondary compounds. Primary constituents include chlorophyll, proteins, and common sugars, while secondary constituents comprise terpenoids, alkaloids, Carbohydrates, flavonoids, and saponins. The secretion of these compounds varies from plant to plant, with some plants producing them in larger quantities and others in minimal amounts (Wagner *et al.*, 2009) [15].

Phytochemical screening was performed at School of engineering and technology Nagaland University. All the materials and laboratory equipment's were present here at the collage.

The sample used for phytochemical screening were different type of Endophytes in the D. *giganteus* bamboos from different parts of Nagaland (Wokha, Kiphire and Longlang)

Reagents used

Table 1: Representation of qualitative experiments performed, test name and reagent used.

Sl.no	Phytochemical	Test	Reagents
1	Terpenoids	Salkowski's Test	Conc. H2SO4 and Chloroform
2	Flavonoids	Sodium hydroxide test	Amino Acid and Conc. Sulfuric Acid
3	Alkaloids	Wagner's test	Iodine in potassium iodide
4	Carbohydrates	Benedict's test	copper sulfate, sodium citrate, and sodium carbonate

Following standard procedures were used for different types of tests: Test for terpenoids: Terpenoids, also known as terpenes, are volatile organic compounds responsible for the fragrance of many plants and flowers (Harbon & Baxter *et al.*, 1995). These compounds exhibit significant pharmacological activities, including anti-cancer, anti-viral, and anti-bacterial properties (Dewick *at el.*, 2009) [14].

Terpenoids can induce apoptosis, inhibit angiogenesis, and modulate immune responses to prevent cancer cell growth (Thoppil, Bishayee 2011) [15]. They inhibit viral replication

and interfere with the viral lifecycle, reducing infection severity. Additionally, terpenoids disrupt bacterial cell walls and membranes, inhibiting bacterial growth and serving as potential antibiotic alternatives. (Burt *at el.*, 2004) [17].

It begins with the addition of 100 µL of the plant extract to a clean test tube, followed by the careful incorporation of 2 mL of chloroform and subsequent introduction of 3 mL of concentrated sulfuric acid in a slow, controlled manner to prevent excessive heat generation, ensuring thorough mixing by gentle swirling to facilitate the reaction between the components. The test tube is then held vertically to allow for careful observation at the interface of the chloroform and sulfuric acid layers, where the formation of a distinct reddish-brown ring indicates the presence of terpenes within the extract.

Test for flavonoids

Flavonoids are especially known for their potent antioxidant activities, which play a significant role in promoting cardiovascular health and preventing cancer (Harbone *et al.*, 1995). These compounds help neutralize free radicals, thereby reducing oxidative stress and inflammation. In addition to their antioxidant properties, flavonoids exhibit a range of other health benefits, including antihistamine effects, antimicrobial activity, memory enhancement, anti-diabetic properties, and mood-boosting capabilities. Their diverse pharmacological actions make flavonoids valuable in both nutritional and therapeutic contexts

To confirm the presence of flavonoids in a sample, 100 µL of the sample suspected to contain flavonoids is carefully transferred into a clean test tube, followed by the addition of 2 mL of ammonia solution, and then 1 mL of concentrated sulfuric acid is slowly and cautiously added down the side of the tube to prevent excessive heat generation, ensuring thorough mixing by gentle swirling; the appearance of a distinct yellow coloration or precipitate at the interface of the solution confirms the presence of flavonoids.

Test for alkaloids

Alkaloids contain one or more nitrogen atoms usually in a heterocyclic ring and are crystalline and non-volatile solids (Dewick *et al.*, 2009) [14]. In plants, they act as reservoirs for protein synthesis and may function as protective substances against animal or insect attacks. Alkaloids are used as anesthetic agents and for reducing headaches and fever.

Wagner's test: It begins with the addition of 100 µL of Wagner's reagent (iodine in potassium iodide) to a test tube containing 100 µL of the sample suspected to contain alkaloids, followed by careful swirling to ensure thorough mixing and allowing the reaction to proceed; the appearance of a distinct brown or reddish precipitate at the interface of the reagent and sample confirms the presence of alkaloids.

Test for carbohydrates

Carbohydrates are essential for life as they form a major portion of our diet, serving as vital body fuels and the primary source of energy for metabolic processes. They are crucial for the proper functioning of our bodies, providing the necessary energy to sustain physical activities and maintain physiological functions (Dewick *et al.*, 2009) [14]. Additionally, carbohydrates are integral to the structural materials of all living cells, playing a key role in cell structure and function, as well as in the storage and transport of energy within biological systems.

Benedict's test: Begins with the addition of 100 µL of Benedict's reagent (containing copper sulfate, sodium citrate, and sodium carbonate) to 1 mL of the sample suspected to contain carbohydrates in a clean test tube, followed by gently heating the mixture in a water bath or using a heating mantle at approximately 80-100°C for 3-5 minutes, during which the reducing sugars present in the sample react with the copper ions in the reagent, resulting in the formation of a varying intensity of orange-red to reddish-brown precipitate, the intensity of which correlates with the concentration of reducing sugars, thereby providing a qualitative and semi-quantitative indication of the presence of carbohydrates.

Antimicrobial screening

Antimicrobial screening is a crucial process in pharmaceutical and medical research aimed at evaluating the ability of substances to inhibit the growth of microorganisms, including bacteria, fungi, and viruses. This screening involves a series of assays designed to assess the antimicrobial potential of natural or synthetic compounds, with the goal of identifying effective agents for the development of new antimicrobial drugs or natural products-based therapies (Palombo *et al.*, 2011) [19]. The screening methods typically include determining the minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration (MBC/MFC), and evaluating the spectrum of antimicrobial activity against a panel of standard microorganisms (Cushnie *et al.*, 2005) [20]. Antimicrobial screening plays a pivotal role in the discovery and development of novel antimicrobial agents to combat infectious diseases and address antibiotic resistance (Cowan *et al.*, 1999) [21].

The samples were collected from leaves and brush of different type of Endophytes in the D. *giganteus* bamboos from different parts of Nagaland (Wokha, Khipheri and Longland) and was isolated and cultured for 10 days before starting antimicrobial screening.

Requirements

Kanamycin, DMSO, bacterial strain; Staphylococcus *aureus* (Newman) and Escherichia coli (TLTE11) Procure from NU Lumami headquarter, Ethyl Acetate, 70%, Ethanol, 3% Sodiumhypochloride, petri plates, micropipette, laminar air flow, incubator, burner and centrifuge tube.

Table 2: Representation of Isolated Endophytic Fungi

Sl.no	*D. giganteus*	Isolated From
1	Isolate 1	Branch
2	Isolate 2	Leave and Branch
3	Isolate 3	Branch

Procedure for Antimicrobial Screening

Preparation of Extracts: Plant materials were dried, powdered, and extracted using Ethyl Acetate. Extracts were concentrated under reduced pressure to yield a crude extract. The extracts were resuspended with DMSO for further analysis.

Preparation of Media

Nutrient agar was prepared and sterilized at 121°C for 15 minutes. Sterilized media was poured into petri plates and allowed to solidify.

Inoculation
Overnight cultures of bacterial strains (Staphylococcus *aureus* and Escherichia coli) were prepared. 100 µL of each bacterial culture was evenly spread onto the nutrient agar plates.

Application of Extracts
Using the agar well diffusion method, wells of 6 mm diameter were aseptically punched into the agar plates. 50 µL of the plant extract was added to each well. Kanamycin (50 µg) served as the positive control, and DMSO served as the negative control. The plates were incubated at 37 °C for 24 hours.

Observation
Zones of inhibition around each well were measured to determine antimicrobial activity. Results were compared against the positive (Kanamycin) and negative (DMSO) controls.

Result
Phytochemical Screening
The presence of various phytochemicals in the extracts from D. *giganteus* was determined through standard qualitative tests.

Table 3: Phytochemical screening results

Sl. No	Alkali Test	Flavonoids	Alkaloids	Trepenoids	Carbohydrate
1	*D. giganteus* Isolate 1	-	-	+	-
2	*D. giganteus* Isolate 2	-	-	+	+
3	*D. giganteus* Isolate 3	-	+	+	+

Present,+; Absent,-;

The phytochemical analysis revealed that terpenoids were consistently present in all isolates, while flavonoids and carbohydrates were only detected in specific samples.

Table 4: Represents the result of D. *giganteus* of Isolation 1

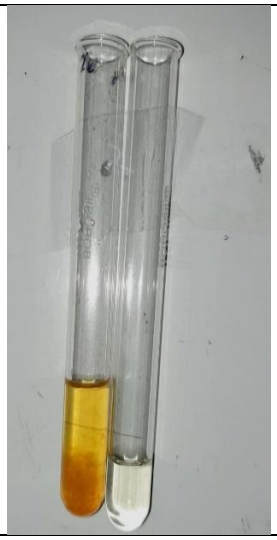
Alkali Test	Flavonoids	Alkaloids	Trepenoids	Carbohydrate
D. giganteus Isolate 1				

Table 5: Represents the result of D. *giganteus* of Isolation 2

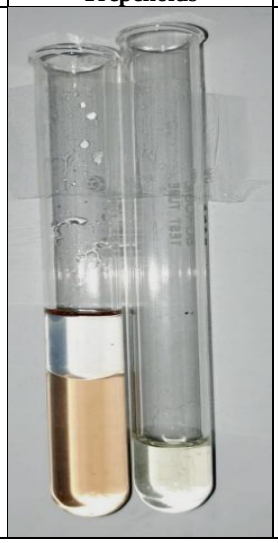
Alkali Test	Flavonoids	Alkaloids	Trepenoids	Carbohydrate
D. giganteus Isolate **2**				

Table 6: Represents the result of D. *giganteus* of Isolation 3

Alkali Test	Flavonoids	Alkaloids	Terpenoids	Carbohydrate
D. giganteus Isolate 1				

Conclusion for Phytochemical Screening

The phytochemical screening of endophytic fungi isolated from *Dendrocalamus giganteus* revealed the presence of a diverse array of bioactive compounds, including terpenoids, alkaloids, flavonoids, and carbohydrates. This diversity of phytochemicals reflects the complex biochemical capabilities of endophytic fungi and highlights their role in the ecological interactions within the plant. The identification of these compounds demonstrates the potential of endophytic fungi as a rich source of natural substances with significant biological activities.

Isolation of Endophytic Fungi: The isolation process successfully identified multiple endophytic fungi from *Dendrocalamus giganteus*. These isolates were found to produce a variety of phytochemicals with known therapeutic potentials and exhibited substantial antimicrobial properties. This study underscores the ecological and pharmaceutical significance of endophytic fungi in bamboo, suggesting that they are promising candidates for the development of new natural products for medicinal and agricultural applications. The isolation and characterization of these fungi pave the way for future research aimed at harnessing their bioactive compounds for drug discovery and biocontrol solutions.

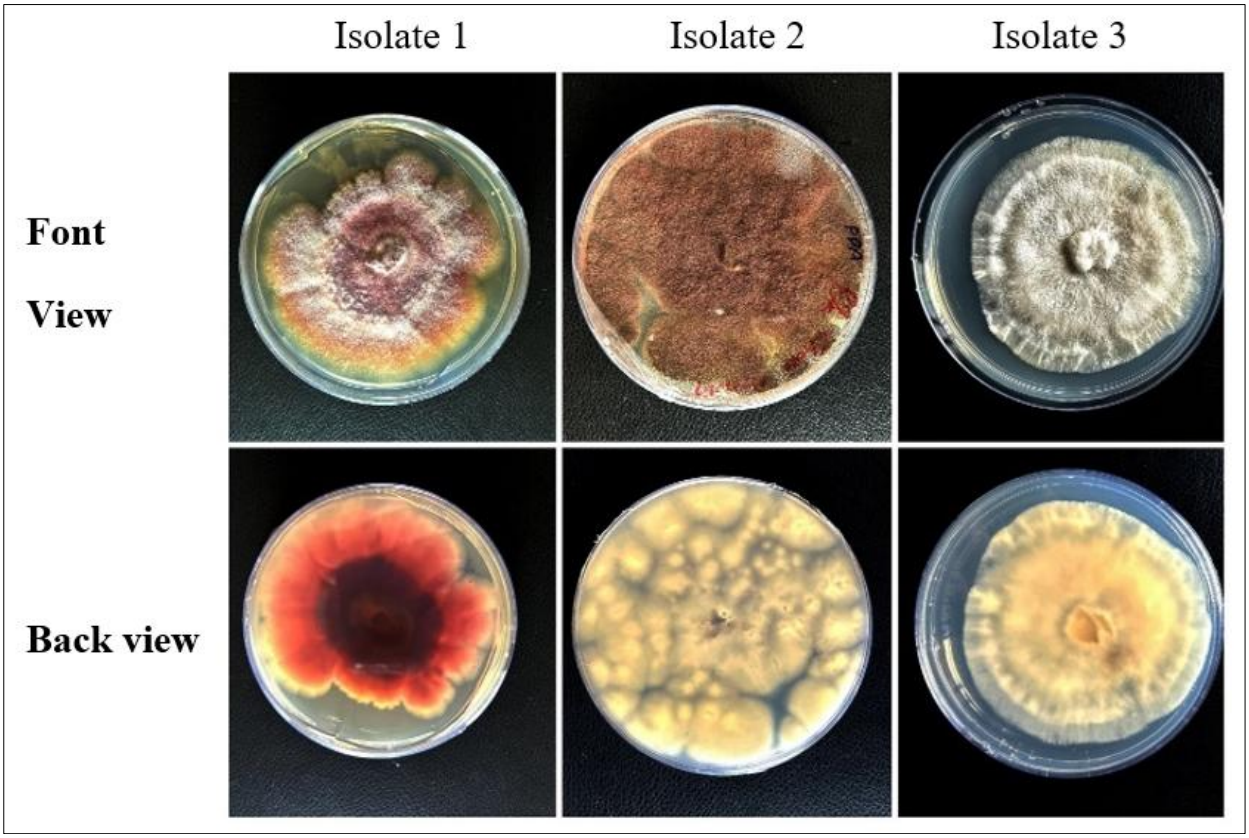


Fig 2: 10 Days culture of Endophytes isolated from *Dendrocalamus giganteus* bamboo

Antimicrobial Screening Result: The antimicrobial activity of the extracts from *D. giganteus* was assessed against *Staphylococcus aureus (Newman*) and *Escherichia coli (TLTE11*) using the agar well diffusion method. The zones of inhibition were measured and compared to both positive *(Kanamycin*) and negative (DMSO) controls.

Table 7: Represents the zone of Inhibition of the antimicrobial

Sl. No	Sample(100mg Extract)	*S.Aureus* (*Newman*)mm	*E.Coli* (*TLTE11*) mm	Negative Control (DMSO)mm	(50µg *Kanamycin*) *S.Aureus* (Newman)mm	(50µg *Kanamycin*) *E.Coli* (TLTE11) mm
1	D. *giganteus* Isolation 1	10mm	14mm	-	20mm	15mm
2	D. *giganteus* Isolation 2	8mm	10mm	-	20mm	15mm
3	D. *giganteus* Isolation 3	8mm	10mm	-	20mm	15mm

These results indicate that the extracts from D. *giganteus* exhibited moderate antibacterial activity against both *Staphylococcus aureus and Escherichia coli.* The inhibition zones ranged from 8±10 mm for *S. aureus* and 10±14 mm for *E. coli*, compared to 20 mm±15 mm respectively for the positive control *Kanamycin.*

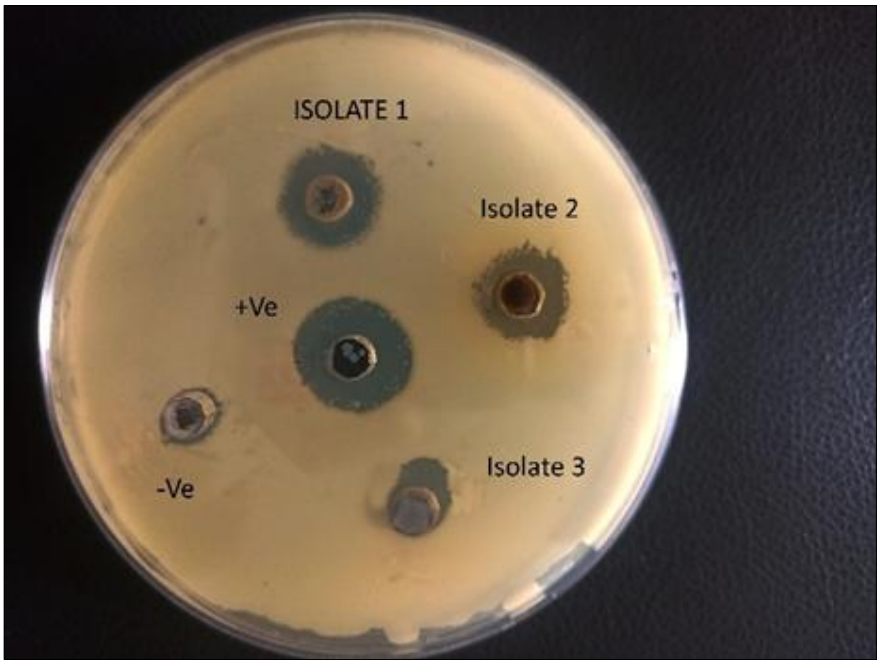


Fig 3: Endophyte extract from D. *giganteus* (Isolation 1,2 and 3) against *E.coli. Where Kanamycin* is positive control(50µg) and DMSO as negative control.

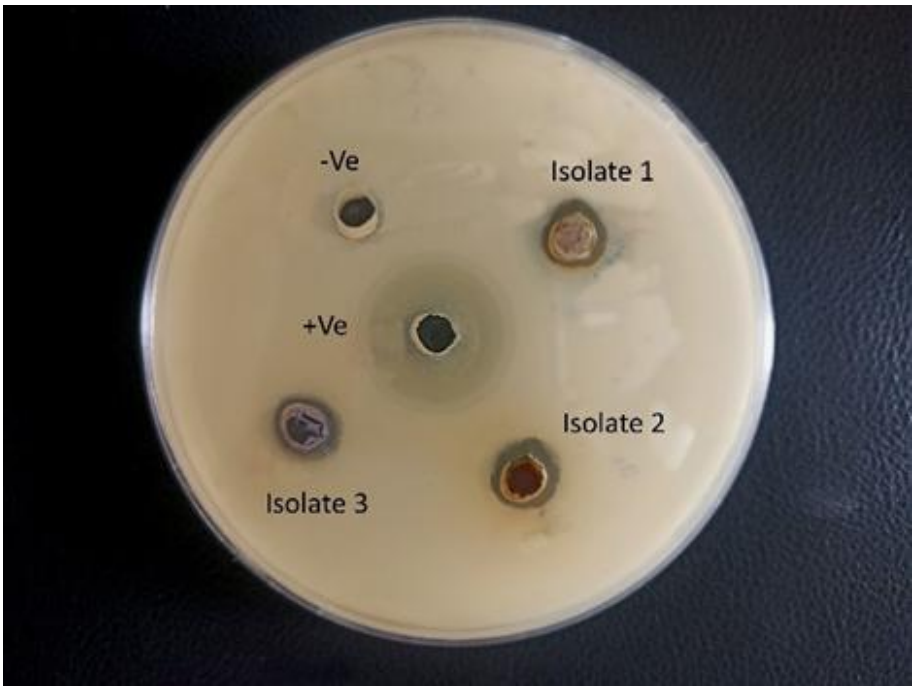


Fig 4: Endophyte extract from D. *giganteus* (Isolate 1,2 and 3) against *S.aureus. Where Kanamycin* is positive control (50µg) and DMSO as negative control

Conclusion for Antimicrobial Screening

Based on the results of the antimicrobial screening, the extracts from *Dendrocalamus giganteus* demonstrated moderate antibacterial activity against both *S.aureus* and *E. coli*. The observed inhibition zones (8±10 mm for *S. aureus* and 10±14 mm for E. coli) suggest a noticeable but limited effectiveness compared to the positive control, Kanamycin (20 mm±15 mm). These findings indicate that D. *giganteus* extracts contain compounds with potential antibacterial properties, albeit with a lesser potency than the standard antibiotic.

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