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Article

In Vitro Activity of Isolated Bioactive Metabolites from Endophytic Fungus Associated with *Aegiceras corniculatum*

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Abstract: A vast and unexplored source of diverse and unique compounds and biological properties is provided by the mangrove fungi. The primary goal is to investigate the biological effects of secondary bioactive compounds produced by endophytic fungi that reside in *Aegiceras corniculatum* bark, focusing on their antioxidant, alpha-glucosidase inhibitory, and antimicrobial properties. Seven distinct strains of endophytic fungi were isolated, of which three particular strains (ACSF-1, ACSF-3, and ACSF-5) were selected for further examination. These strains were cultivated in potato dextrose broth (PDB) and underwent extraction using dichloromethane (DCM) and ethyl acetate (EtOAc). In the DPPH assay, the fraction ACSF-3 of the DCM showed a good IC_{50} value of 239.88 µg/mL, whereas the ascorbic acid IC_{50} was 15.985 µg/mL. Additionally, the crude extract ACSF-3 exhibited the highest levels of total phenolic content (89.89 mg GAE/g), total flavonoid content (288.52 mg QE/g), and total tannin content (53.85 GAE/g). To evaluate antihyperglycemic activity, the ACSF-3 n-Hexane fraction, which showcased the highest efficacy with a value of 0.91 µg/mL. The extracts of ACSF-1 and ACSF-3 demonstrated significant zones of inhibition against Escherichia coli, with sizes reaching up to 16 and 12 mm, respectively, and ACSF-5 displayed the highest zone of inhibition against *Staphylococcus aureus*.

Keywords: *Aegiceras corniculatum*; endophytic fungus; antioxidant; antimicrobial; antihyperglycemic

1. Introduction

Endophytes are microbes that present inside the plant tissues and do not produce any harm to the plants for a significant portion of their life cycle [1]. Endophytic fungi are inherent elements of the plant micro-ecosystem that exert a beneficial influence on the physiological functions of the host plant through various mechanisms such as synthesis of hormones, biosynthesis, and acquisition of nutrients to support plant growth and development. In addition, these secret metabolites aid in the plant's defense against herbivores and pathogens, and the enhancement of the plant's ability to adapt to abiotic stressors [2]. In reciprocation, plants provide both a habitat and nourishment for these endophytic fungi [3]. Microorganisms that are endophytic a reservoir of unique secondary metabolites that can serve as a potential source of drug discovery across diverse therapeutic areas, including antiarthritic, antimicrobial, anticancer, antidiabetic, anti-insect, and immunosuppressant activities [4,5].

Aegiceras corniculatum, a member of the Myrsinaceae family, is primarily a diminutive shrub that thrives in the wetlands of Asia and Australia. It possesses the ability to withstand an extensive array of environmental conditions, thereby enabling its presence in a diverse range of tidal habitats. The species exhibits resilience in the face of varying degrees of salinity and exposure to sunlight, allowing it to prosper in an assortment of soil types. Frequently, it can be observed forming a dense sub-canopy along the periphery of tidal creeks and river margins. However, in coastal mangrove ecosystems, it predominantly flourishes along the inland boundaries [6].

The members of the family Myrsinaceae characteristically show presence of secondary metabolite. The identification and characterization of bioactive metabolites from endophytic fungi inhabiting the tissues of *Aegiceras corniculatum* have drawn substantial attention in recent times because of their potential applications across diverse domains such as medicine and agriculture. From several researches, it is obvious that *A.*

corniculatum possesses anti-tuberculosis, antibacterial, antioxidant, and anti-inflammatory activities [7,8]. In addition, anti-diabetic, effectivity for chronic granulomatous inflammation and arthritis has been proved in animal models [9,10]. However, bioactive metabolites of endophytic fungus associated with *Aegiceras corniculatum* is still create ambiguity. Therefore, we have endeavored to identify the secondary metabolites and their corresponding pharmacological activities, which could potentially aid in future drug discovery.

2. Materials and Methods

2.1. Sample Collection and Isolation of Endophytic Fungi

Fresh barks of *Aegiceras corniculatum* were collected in September, from Mangrove Sundarbans, Bangladesh. The samples were kept in disinfected individual plastic bags and transferred to the laboratory within 24–48 h. The gathered plant specimens underwent a thorough cleansing process involving rinsing under flowing tap water, followed by immersion in distilled water. The plants were mounted on paper, and the sample was identified by the experts of the Bangladesh National Herbarium (voucher specimen no. 44815). After cutting into small pieces about 0.5 cm^2, the samples underwent a sterilization process. This involved sequential treatments with 75% ethanol (1 min), 5% sodium hypochlorite (3 min), and another round of 75% ethanol (30 s), followed by meticulous rinsing with sterile distilled water [11]. Further, Sterilized specimens of tissues were spread out over Potato Dextrose Agar medium in Petri dishes horizontally along with Chloramphenicol (150 µg/mL). After that, the plates were placed in incubation at room temperature until fungal growth appeared (1–3 week). From the first culture plate, a minute amount of the fungus was carefully extracted from the distinct colony and subsequently introduced into a Petri dish infused with potato dextrose culture media. In order to prevent bacterial contamination, Lactic Acid or Chloramphenicol may be incorporated into the medium. Once a pure culture is achieved, the fungus is cultivated for 5 to 10 days. The storage procedure may then commence.

2.2. Identification and Morphological Characterization of Endophytic Fungi

The identification of the endophytic fungi was carried out through the analysis of their morphological characteristics. This involved a comprehensive analysis the colony's characteristic, including the morphology, coloration of the aerial hyphae, growth rate, margin features, surface texture, and the presence of spores or reproductive structures. To induce sporulation, the mycelia of the endophytic fungi were placed on sterilized segments of host plants. Following this, the spores and mycelia were extracted from the refined colonies, deposited onto glass slides, and subjected to staining with lactophenol cotton blue (1% *v*/*v*). The prepared slides were then scrutinized under a light microscope for further examination (Olympus, Bartlett, TN, USA). The taxonomic classification was determined utilizing a handbook dedicated to the identification of fungi [12]. Fungi are primarily recognized through meticulous scrutiny of their morphology and distinctive traits. In the technique, fungi are grown on a slide directly which was prepared by a layer of agar. This approach eliminates the necessity of transferring fungus from a culture plate to a specific slide, reducing the risk of damage to vital identifying features, particularly the spore-bearing structures. The isolates were subjected to a thorough identification process involving a meticulous review conducted 2–3 times, in conjunction with proper reference. The identification of the isolates yielded the following results: ACSF-1, ACSF-3, and ACSF-5, and the yields were 1.7 g, 2.1 g, and 2 g, respectively.

2.3. Extraction of Fungus and Preparation of Crude Extract

Potato dextrose broth media was prepared through the addition of water (24 mg/mL) to a dehydrated product. Sterilized Potato dextrose broth media, Scott bottle, Erlenmeyer flask as well as its cotton cap, and Cork borer (maintained temperature of 121 °C and a pressure of 15-lbs/sq inch for 15 min) placed in a laminar hood and UV light 30 min before working. The endophytic fungi were subsequently cultivated in potato dextrose broth (PDB) utilizing a cubic shaker operating at 150 rpm, with an incubation period of 21 days at a temperature of 28 ± 2 °C. During the phase of separation, the mycelium was meticulously separated from the broth by means of cotton filtration to ensure aseptic conditions. In a separatory funnel where n-hexane was added in a 2:1 ratio to facilitate defatting, the broth had been subsequently relocated [13]. The upper layer of n-hexane which contained the fatty material was then separated from the separatory funnel. Following this, the secondary metabolite was extracted from the defatted broth using dichloromethane (DCM) or ethyl acetate. Lastly, with the help of a rotary evaporator at a temperature of 37 °C the extracted liquid was evaporated [14].

2.4. Screening of Antioxidant Activity

2.4.1. Screening of DPPH Activity

The DPPH scavenging assay was conducted in accordance with an established procedure, with specific modifications to suit the experimental requirements [15]. The endophytic fungal extracts which was dissolved in methanol at 1 mg/mL, were used to prepare the stock solutions. It was then serially diluted into a 96-well plate along with methanol so that concentrations lie from 1000 to 3.90 µg/mL range can be achieved. Then, we added 100 µL of 0. 2 mM DPPH solution (Sigma-Aldrich, St. Louis, MO, USA) to each well and incubated the plates in the dark at 25 °C for 30 min, covered with a lid and aluminum foil. We measure absorbance at 517 nm using a microplate reader and some standards like ascorbic acid (Merck, Darmstadt, Germany) and butylated hydroxytoluene (BHT) (Merck, Indianapolis, India) were used. Each sample was monitored thrice.

2.4.2. Quantification of Total Phenol Content (TPC)

The quantification of total polyphenol content in the endophytic fungal extract was carried out following a standardized protocol employing Folin-Ciocalteu's reagent [16]. In this procedure, 200 µL of each extract solution (1 mg/mL) was used along with 200 µL of Folin-Ciocalteu's reagent from Sigma-Aldrich in the United States and 2 mL of distilled water. The mixing was continued and 1.6 mL of a 7.5% Na_2CO_3 solution was added meanwhile. After that the mixture was placed in the darkness at room temperature for 90 min for incubation. The measurement of absorbance was taken at 760 nm and distilled water was used as a blank in a spectrophotometer. Finally, we calculated total polyphenol content by using a standard calibration curve which was generated with six gallic acid concentrations from 6.25 to 200 µg/mL). The results were demonstrated as gallic acid equivalent (GAE) in µg mg^{-1} of dried fungal extract. Each sample was observed thrice.

2.4.3. Determination of Total Flavonoid Content (TFC)

We have followed colorimetric method where aluminum chloride was used to ascertain the flavonoid content of the given sample [17] with some possible modifications. In short, to assess the flavonoid amount in fungal extract (1 mg/mL), we took 300 µL of the extract which was added to 3.4 mL of 30% methanol, and 150 µL sodium nitrite ($NaNO_2$) solution 5% concentration. Further, we combined Aluminum chloride ($AlCl_3$) 150 µL solution of 10% concentration, and 1 mL of 1 M sodium hydroxide (NaOH) solution. After thorough mixing, the absorbance had been measured at 510 nm weave length using a spectrophotometer where distilled water was taken as the blank. For calculating the final data, we used a standard calibration curve in which six concentrations (6.25–200 µg/mL) of quercetin was added, and the flavonoid concentration was measured and expressed as quercetin equivalents (QE) in µg/mg of fungal extract that had been dried. Each sample was tested three times.

2.4.4. Determination of Total Tannin Content (TTC)

The tannin content test procedure comprised several sequential steps [18]. Initially, separate test tubes were used to hold 0.1 mL of standard solutions at varying concentrations (0.5, 0.4, 0.3, 0.2, 0.1 mg/mL) and 0.1 mL of the extract sample. Subsequently, each test tube received distilled water in the amount of 7.5 mL. Additionally, we took FC reagent in the amount of 0.5 mL was introduced in test tube. Then we added 1 mL of 35% Na_2CO_3. Following the addition of 0.1 mL standard solution or extract sample to separate test tubes, 7.5 mL water specially distilled water was added to each tube. Subsequently, FC reagent had been taken 0.5 mL along with 1 mL Na_2CO_3 in which the concentration was 35%, and the solutions were diluted to with distilled water of 10 mL. After vertexing for 15 s, the test tubes were kept 30 min at the room temperature condition. The measurement of absorbance of the solutions had then measured at the wavelength of 725 nm. A blank was prepared following the same steps as above, excluding the addition of gallic acid and the sample. It's important to note that a blank was prepared by replicating the aforementioned steps, excluding the inclusion of gallic acid and the sample in the process.

2.5. Alpha-Glucosidase Inhibitory Activity

The experiment was conducted in accordance with a previously established procedure with certain alterations [19]. At first, we collected endophytic fungal extracts which was then dissolved in dimethyl sulfoxide (DMSO) and prepared stock solutions at the amount of 8 mg/mL. Subsequently, we took each stock solution of 50 µL which was aimed at serial 2 × dilution in a 96-well plate using potassium phosphate buffer solution (PBS) of 100 mM with a pH of 6.8. This process yielded a concentration range from 4 to 0.031 mg/mL. Subsequently, each well was enriched with α-glucosidase enzyme (Sigma-Aldrich, United States) solution 20 µL, measuring 0.4 unit/mL. The samples were then incubated at 37 °C for 10 min, after which 4-nitrophenyl α-D-glucopyranoside (pNPG) (Sigma-

Aldrich, United States) (5 mM) was introduced at the amount of 10 µL. The mixtures were further incubated at one significant temperature which was 37 °C for an additional 30 min. The conclusion of the reaction was achieved by the addition of 50 µL of a solution of sodium carbonate (Na_2CO_3) with a concentration of 0.2 M. To quantify the absorption, a microplate reader was employed at a wavelength of 405 nm. Negative control was established employing SDW, while the standard was executed with acarbose. Employing an established method [20] mixture was remained at the same temperature before like 37 °C for 20 min within a 96-well plate for each concentration. Next, to each well, we added 20 µL α-glucosidase enzyme solution with possessing of 0.4 µ/mL concentration. The plates were subsequently mixed thoroughly and subjected to re-incubation for a duration of 10 min at a temperature of 37 °C. Following the incubation process, the reaction was ceased by incorporating 50 µL of a solution consisting of 0.2 M sodium carbonate (Na_2CO_3) into each well. The measurement of optical density (OD) was conducted at a wavelength of 405 nm using a microplate reader. To ascertain the initial velocity of the reaction, the OD value was correlated with time for different concentrations of the substrate. The determination of Km and Vmax values was accomplished through the utilization of Lineweaver-Burk and Michaelis-Menten plots. The experiments were performed on three separate occasions.

2.6. Test for Antimicrobial Activity

For determination of antimicrobial activity, we followed microdilution assay of broth, hence achieved minimum inhibitory concentrations (MICs) for each fungal extract, adapting established methodologies for endophytic fungi [21]. At the beginning, one fresh stock solution, total amount 6 mg/mL was prepared by dissolving the extracts (ACSF-1, ACSF-3, ACSF-5) in acetone. In addition, this stock solution went through serial dilution process with water which was sterilized as well as distilled. It allowed to achieve different concentrations stared from 3 mg/mL and end up with 0.005 mg/mL. Standardized bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella enterica* suspensions were inoculated into wells containing the extract and incubated under specific conditions. After incubation, MICs were determined by introducing resazurin dye (Sigma-Aldrich, United States), where a blue color signified the absence of visible microbial growth. After incubation, we checked for the minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC). These concentrations were determined as the lowest where we saw fewer than one visible colony. To compare and validate our results, we included positive controls like the standard antibiotic Ciprofloxacin. The presence of more than one colony indicated significant effect called bacteriostatic/fungistatic effect means it cannot kill the bacteria whereas suppress their growth. The whole test was repeated thrice for accuracy.

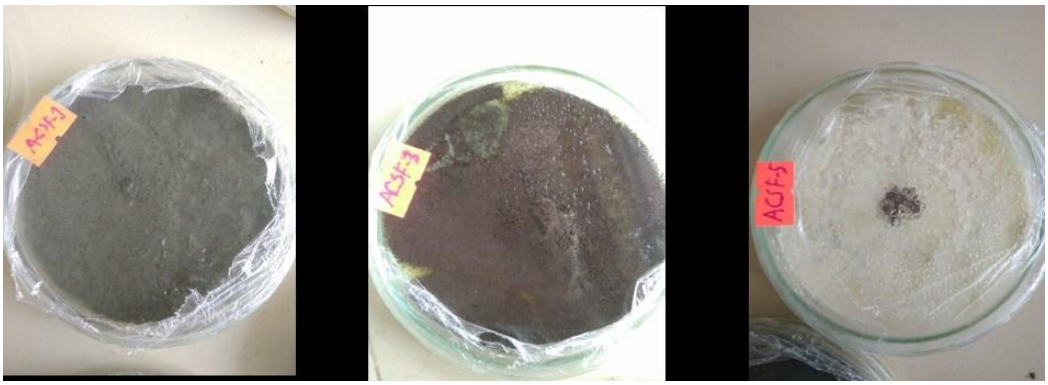
2.7. Statistical Analysis

GraphPad Prism 8.0 (GraphPad Software by Dotmatics, Boston, USA) as used to perform statistical analysis for the antioxidant experiment. We calculated mean and SEM for data calculation in terms of each test parameter. $y = mx + c$ equation has been employed for IC_{50} and p value 0.05 has been considered as statistical significance.

3. Results

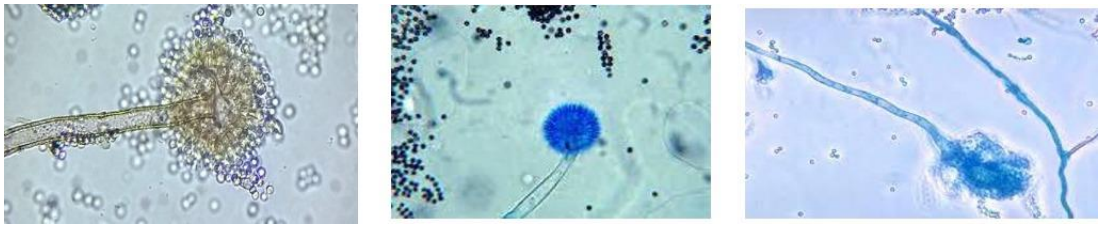
3.1. Isolation, Identification and Extraction

Seven strains of endophytic fungi were procured from the bark of the mangrove plant, *Aegiceras corniculatum.* Among the seven strains the 3 were isolated and further extracted. The identity of the isolates is: ACSF-1, ACSF-3, ACSF-5 (Figures 1 and 2). After isolation, these three strains were extracted and so achieved the crude extract through the solvent partitioning system. They were fermented on potato dextrose broth (PDB) and extracted using dichloromethane (DCM) and ethyl acetate (EtOAc) after defatting by using n-hexane.



a **b** **c**

Figure 1. Identity of the isolates (ACSF-1, ACSF-3 and ACSF-5).



a **b** **c**

Figure 2. Microscopic Identification of ACSF-1, ACSF-3 and ACSF-5.

3.2. DPPH Free Radical Scavenging Activity

The methanolic extract of bark of the *Aegiceras corniculatum* and extracted endophytic fungi demonstrated DPPH free radical scavenging activity which was really significant. The well-known standard antioxidant, ascorbic acid, exhibited an IC_{50} value of approximately 15.985 µg/mL, confirming its efficacy as a reference compound for antioxidant activity. The crude extracts and fractions of ACSF-1, ACSF-3, and ACSF-5 exhibited varying degrees of DPPH free radical scavenging activity, with IC_{50} values of 156.31 µg/mL, 87.48 µg/mL, and 202.25 µg/mL, respectively (Figure 3). Fractionation of the ACSF-3 extract yielded n-Hexane, DCM, and Ethyl acetate fractions, with IC_{50} values of 213.79 µg/mL, 239.88 µg/mL, and 72.44 µg/mL, respectively (Figure 3). These results suggest that different fractions may possess distinct antioxidant potential.

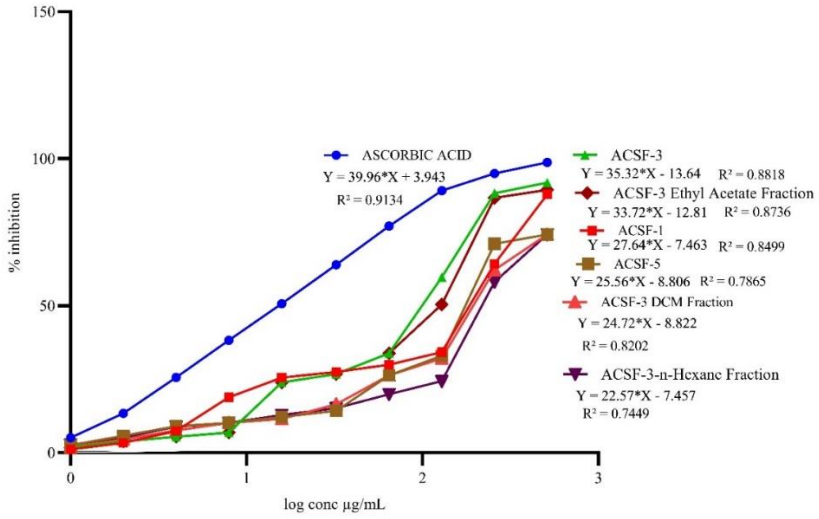


Figure 3. DPPH scavenging activity of endophytic fungal extracts.

3.3. Total Polyphenolic Content

ACSF-1 Crude Extract (ACSF-1) possess Total Phenolic Content Approximately 68.77 mg GAE/g. In addition, n-Hexane Fraction, DCM (Dichloromethane) and Ethyl Acetate Fraction (ACSF-1) exert Total Phenolic Content approximately 5.29 mg GAE/g, 9.89 mg GAE/g and 49.67 mg GAE/g respectively (Figure 4). Moving on to ACSF-3, the crude extract shows a remarkably high Total Phenolic Content, measuring approximately 89.89 mg GAE/g. However, the n-Hexane Fraction of ACSF-3 exhibits a lower Total Phenolic Content, approximately 8.17 mg GAE/g. The DCM and Ethyl Acetate Fraction of ACSF-3 demonstrates an intermediate Total Phenolic Content, measuring about 19.19 mg GAE/g and 65.11 mg GAE/g (Figure 4). The crude extract from ACSF-5 was found to possess a Total Phenolic Content of approximately 27.45 mg GAE/g. In contrast, the n-Hexane Fraction of ACSF-5 exhibited a notably lower Total Phenolic Content, measuring approximately 6.12 mg GAE/g. Moving forward, the DCM (Dichloromethane) Fraction of ACSF-5 displayed an intermediate Total Phenolic Content, measuring about 11.54 mg GAE/g. Finally, the Ethyl Acetate Fraction of ACSF-5 demonstrated a relatively higher Total Phenolic Content, approximately 23.88 mg GAE/g (Figure 4).

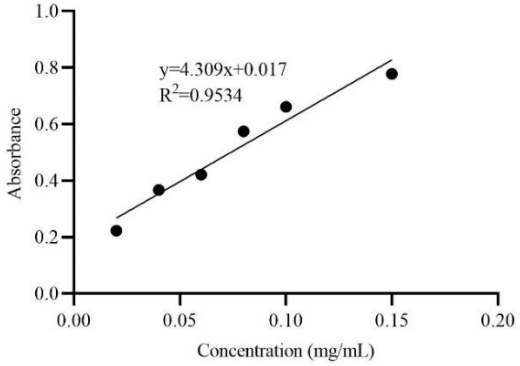


Figure 4. Total Phenolic Content of endophytic fungal extracts based on gallic acid standard calibration curve.

3.4. Total Flavonoid Content

The analysis across ACSF-1, ACSF-3, and ACSF-5 fractions, along with their respective crude extracts, reveals distinct variations. Within the DCM (Dichloromethane) fractions, ACSF-3 exhibits the highest flavonoid content at approximately 57.6 mg QE/g, followed by ACSF-1 with about 29.31 mg QE/g, and ACSF-5 with approximately 22.1 mg QE/g (Figure 5). Conversely, the n-Hexane fractions show lower flavonoid content, with ACSF-3 having the highest at approximately 8.76 mg QE/g, followed by ACSF-1 with about 5.56 mg QE/g, and ACSF-5 at approximately 3.12 mg QE/g (Figure 5). Notably, the Ethyl Acetate fractions consistently demonstrate relatively higher flavonoid content across all three samples, with ACSF-3 having the highest at approximately 131.96 mg QE/g, followed by ACSF-1 at about 94.92 mg QE/g, and ACSF-5 with approximately 76.2 mg QE/g (Figure 5). Additionally, the crude extracts of ACSF-1, ACSF-3, and ACSF-5 exhibit substantial flavonoid content, measuring approximately 180.8 mg QE/g, 288.52 mg QE/g, and 116.43 mg QE/g, respectively. This comprehensive summary encompasses flavonoid content variations within the DCM, n-Hexane, and Ethyl Acetate fractions, along with the crude extracts of ACSF-1, ACSF-3, and ACSF-5.

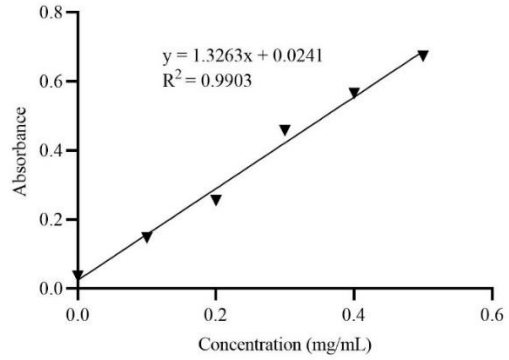


Figure 5. Total Flavonoid Content of endophytic fungal extracts based on quercetin standard calibration curve.

3.5. Total Tannin Content

Across ACSF-1, ACSF-3, and ACSF-5, the tannin content varies within the different fractions. In ACSF-1, the crude extract exhibits a tannin content of approximately 23.63 mg GAE/g, while the DCM fraction measures about 7.78 mg GAE/g, and the Ethyl Acetate fraction shows around 15.23 mg GAE/g (Figure 6). For ACSF-3, the crude extract has a tannin content of approximately 53.85 mg GAE/g, with the DCM fraction measuring about 14.69 mg GAE/g, and the Ethyl Acetate fraction showing approximately 45.6 mg GAE/g (Figure 6). Finally, in ACSF-5, the crude extract demonstrates a tannin content of approximately 38.05 mg GAE/g, with the DCM fraction measuring about 9.24 mg GAE/g, and the Ethyl Acetate fraction showing approximately 31.23 mg GAE/g (Figure 6).

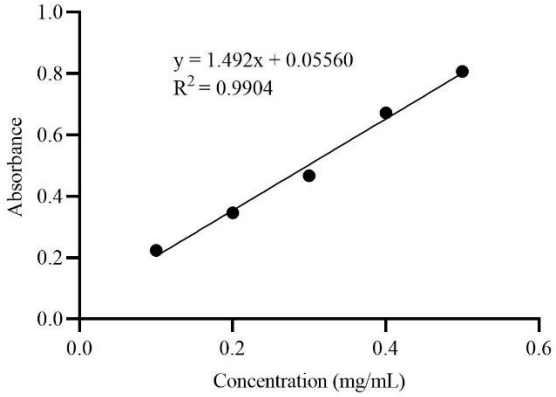


Figure 6. Total Tannin Content of endophytic fungal extracts based on standard curve.

3.6. Antimicrobial Activity

In our antimicrobial susceptibility testing, we assessed the inhibitory effects of different extracts and fractions derived from endophytic fungi against various bacterial strains. The standard antibiotic, ciprofloxacin (30 µg/disc), exhibited robust inhibitory activity with zone diameters ranging from 25 to 30 mm against the tested bacterial strains (Figure 7). Among the fractions of ACSF-1, the crude extract at 250 µg/disc displayed modest inhibition, with zone diameters ranging from 6 to 9 mm against the bacterial strains (Figure 7). When the concentration was increased to 500 µg/disc, the inhibitory effects improved, with zone diameters ranging from 11 to 16 mm. The n-Hexane fraction showed limited inhibitory activity, while the DCM fraction and ethyl acetate fraction demonstrated no inhibitory effects against the tested bacteria at both concentrations. Similar trends were observed for ACSF-3 and ACSF-5 fractions. The crude extracts exhibited moderate to mild inhibitory effects. Notably, the ethyl acetate fractions of ACSF-3 and ACSF-5 displayed relatively better inhibitory activity at 500 µg/disc, with zone diameters reaching up to 14.5 mm against some bacterial strains (Figure 7).

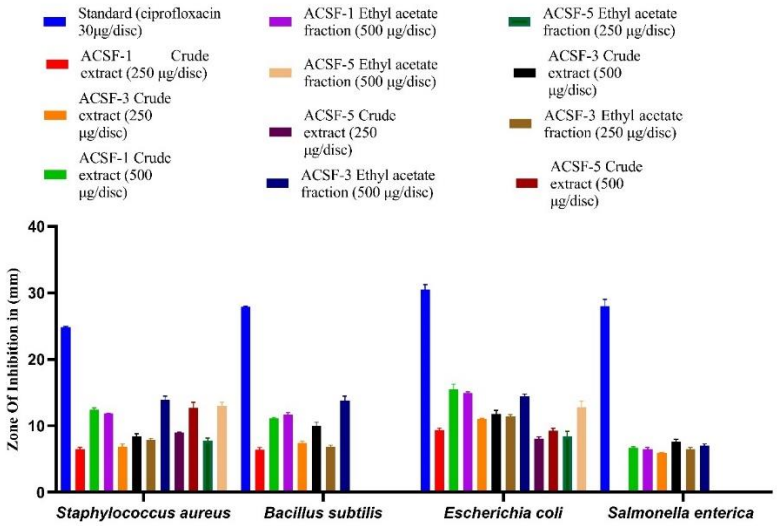


Figure 7. In-vitro antimicrobial activity of ACSF-1, ACSF-3 and ACSF-5 extracts by disc diffusion method.

3.7. α-Glucosidase Inhibitory Activity

The IC_{50} values means the concentration at which half of the total i.e., 50% inhibition of alpha-glucosidase activity occurs, were assessed for various extracts and fractions. Acarbose, a standard antidiabetic agent, exhibited an IC_{50} value of 0.413 μg/mL, indicating its potent inhibitory effect on alpha-glucosidase (Figure 8). Among the crude extracts, ACSF-1 displayed an IC_{50} value of 0.59 μg/mL, while ACSF-3 and ACSF-5 exhibited IC_{50} values of 0.47 μg/mL and 0.67 μg/mL respectively (Figure 8), suggesting their potential as alpha-glucosidase inhibitors. Within the ACSF-3 fractions, the n-Hexane fraction showed 0.91 μg/mL, while the DCM fraction as well as ethyl acetate fraction exhibited values of 0.73 μg/mL and 0.5 μg/mL respectively (Figure 8). These findings indicate variable inhibitory activities of the tested extracts and fractions against alpha-glucosidase, highlighting their potential in managing diabetes.

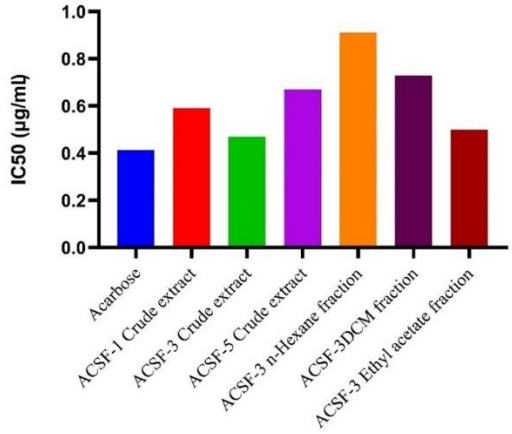


Figure 8. α-Glucosidase inhibitory activity of endophytic fungal extracts.

4. Discussion

The separation and characterization of bioactive adduct from endophytic fungi related to *Aegiceras corniculatum* have provided valuable insights into the potential of these fungal strains as sources of pharmacologically active compounds [22]. Parts of the *Aegiceras corniculatum* plant and the fungi that coexist with it contain new chemicals. These include 3,7-dihydroxy-2,5-diundecylnaphthoquinone, 2-methoxy-3-nonylresorcinol, 5-*O*-ethylembelin, 2,7-dihydroxy-8-methoxy-3,6-diundecyl-dibenzofuran-1,4-dione, and. Three known compounds were also found: 5-*O*-methylembelin, 3-undecylresorcinol, and 2-dehydroxy-5-*O*-methylembelin. 3,7-dihydroxy-2,5-diundecylnaphthoquinone is a derivative of naphthoquinone, and naphthoquinone derivatives show anticancer and antimicrobial activities [23]. In this investigation, we successfully obtained seven strains of endophytic fungi from the bark of *Aegiceras corniculatum*, thus encompassing a diverse array of fungal biodiversity. From this collection, ACSF-1, ACSF-3, and ACSF-5 were specifically chosen for subsequent extraction and assessment of their bioactive properties.

One of the key findings of this study was the remarkable DPPH free radical scavenging activity exhibited by the bark extract which was methanolic of *Aegiceras corniculatum* and extracted endophytic fungi. This indicates the presence of potent antioxidants in both the plant and the associated fungal strains, highlighting their potential in combating oxidative stress-related disorders. The graphical representation of the scavenging activity provides a visual representation of the dose-dependent response, further supporting the antioxidant capacity of these extracts (Figure 2).

The assessment of total polyphenolic content revealed substantial quantities in ACSF-1 crude extract, underscoring its role as a potential source of polyphenolic compounds. The graphical representation of the concentration-absorbance relationship allows for a clear visualization of the phenolic content and its variation across different concentrations.

Additionally, the evaluation of total flavonoid content further elucidated the bioactive potential of the isolated endophytic fungi. The graphical representation aids in understanding the concentration-dependent variation in flavonoid content, which holds significance in various pharmacological applications. The determination of total tannin content added another layer of understanding regarding the chemical composition of the extracts. Tannins, known for their astringent properties and potential health benefits, were found in varying quantities within the tested samples [24,25].

The assessment of antimicrobial activity demonstrated varying inhibitory effects among the extracts and fractions. The importance of antimicrobial assays was reviewed by the following works where researcher was synthesized seven complete lactam Arg_{10}-teixobactin analogues using solid-phase peptide synthesis. The entire lactam ring was obtained by replacing d-Thr_8 with (2R,3S)-diamino-propionic acid. Each analogue was evaluated with MIC against resistant bacteria to confirm their antimicrobial activity and efficacy. Nle^{11}-containing compounds showed noteworthy activity [26]. Notably, the standard antibiotic ciprofloxacin exhibited strong inhibitory activity, providing a benchmark for comparison (Figure 7). These results suggest the potential of certain extracts and fractions in combating bacterial infections, albeit with varying degrees of efficacy. Non-polar compound(s) may potentially contribute to the manifestation of antibacterial activity in disc diffusion assays, as they may demonstrate an inability to effectively disperse in agar media. In accordance with their investigation, the plant extracts showcased noteworthy minimum inhibitory concentrations (MIC) [8].

Finally, the evaluation of alpha-glucosidase inhibitory activity revealed promising results, indicating the potential of the tested extracts and fractions in managing diabetes. The graphical representation of this inhibitory activity provides valuable insights into the dose-dependent response.

Medicinal flora and their endophytes play a significant role as a valuable reservoir for biologically active substances and secondary metabolites, which constitute a majority of the herbal pharmaceuticals present in existing commercial sector [27].

5. Conclusion

The examination and characterization of biologically active substances from endophytic fungi connected with *Aegiceras corniculatum* present stimulating possibilities for pharmaceutical and medicinal applications. The wide array of biologically active compounds recognized like antioxidants, polyphenols, flavonoids, tannins, antimicrobial agents as well as alpha-glucosidase inhibitors which emphasize the pharmacological potential of these fungal strains. Further investigation is justified to separate and identify particular biologically active compounds and investigate their mechanisms of action, paving the way for the development of innovative therapeutic agents derived from these endophytic fungi. This examination highlights the potential of endophytic fungi linked to *Aegiceras corniculatum* as valuable origins of diverse bioactive compounds, providing a multitude of pharmacological advantages. It contributes notably to our comprehension of the natural substances present within the ecosystem of mangroves.

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