

Comparative Analysis of the Root and Root Bark Extracts of *Syzygium samarangense* by Spectroscopic Characterization and Antimicrobial Effects

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Received: 19.10.2024; Accepted: 24.07.2025; Published: 30.09.2025

Abstract: This research was carried out to compare the extracts of the root and the root bark of *Syzygium samarangense* by spectroscopic characterization and antimicrobial effects. Water, ethanol, and dichloromethane were used for the extraction. A phytochemical screening of the parts was conducted. Total phenolic and flavonoid contents were evaluated. Spectroscopic analyses were used to characterize the extracts. The crude extracts were tested against five bacterial strains: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter cloacae*. The root and root bark were found to contain terpenoids, flavonoids, phenols, saponins, tannins, essential oils, and alkaloids; however, cardiac glycosides were not found. The extracts' respective root and root bark have total phenolic contents of 62.87-115.31 ppm and 61.41-130.53 ppm. The range of total flavonoid content in the root extract is 45.76-104.44 ppm, while the root bark extract has a content of 50.37-142.64 ppm. When all the extracts were compared, the most effective extract was found to be the root bark ethanolic extract with the zone of inhibition of 18.67 ± 3.0 mm, which was against *Staphylococcus aureus*, whilst the least effective was found to be the root dichloromethane extract with a zone of inhibition of 6.0 ± 0.0 mm, which was against *Escherichia coli*. The antimicrobial investigations indicated that the ethanolic and aqueous extracts showed the possibility of inhibiting bacterial growth, and this may contribute to the development of novel, plant-based antimicrobial drugs. According to the research, *Syzygium samarangense* root and root bark extracts may have biological activity and be useful in treating specific infections.

Keywords: *Syzygium samarangense*; root; root bark; spectroscopy; phytochemicals; antimicrobial effects.

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1. Introduction

Medicinal plants are widely used throughout the world to treat a wide range of illnesses. Medications made from medicinal herbs are less expensive, easier to get, and have fewer negative effects than synthetic medications [1]. Alkaloids, flavonoids, and polyphenols are just

a few of the many chemical components found in plant extracts that are rich in bioactive compounds and are essential to the development of new drugs [2]. Extraction of these bioactive compounds is influenced by the type of extraction solvents on both the chemical constituents and biological activities [3]

Many cultures and traditions have long practiced traditional medicine, which has identified particular plants with medicinal qualities. Phytochemical and pharmacological methods are commonly used in drug evaluation, which results in the screening of natural products and the discovery of novel drugs [4-6]. Phytochemicals are biologically active substances that show a range of biological activities, including antimicrobial and antioxidant qualities. They can be found in various plant parts, including bark, leaves, flowers, roots, fruits, and seeds [7,8]. With few or nonexistent side effects, herbal medicines are effective in treating a wide range of illnesses [9].

Currently, some intended diseases cannot be effectively treated with conventional medications due to drug resistance. In addition, the new infections are evolving and posing a threat to humankind. Mukatay et al. [10] screened the antimicrobial activity of the total extract and different fractions of *Artemisia heptapotamica* Poljak, and they exhibited no inhibition of the growth of *Candida albicans*, *C. neoformans*, *Aspergillus fumigatus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Vancomycin-resistant Enterococci* (VRE) at the tested concentrations ranging from 8 to 200 µg/mL.

The decoction of *Syzygium samarangense* root bark has historically been used as an abortifacient and to treat dysentery and amenorrhoea. Root is administered as a diuretic to reduce swelling. In their 2021 study, Khan et al. [11] examine the phytochemical profiles of root and bark extracts from various medicinal plants, focusing on the analysis of essential oils and alkaloids. The article addresses comparative phytochemistry with a systematic exploration of the differences in chemical composition between the root and bark of selected species. Essential oils and alkaloids, two classes of compounds with diverse therapeutic applications, were analyzed for composition and concentration, utilizing methods such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). In addition to profiling the phytochemicals, Li et al. [12] evaluated the bioactivity of the extracts. They focused on antioxidant, antibacterial, and anticancer properties. The study showed that the extracts had significant radical-scavenging activity, indicating strong antioxidant potential. Additionally, some *Syzygium* extracts demonstrated notable antibacterial activity, which supports their traditional use in treating infections. The authors also report preliminary evidence of anticancer potential, though they acknowledge the need for more detailed mechanistic studies. Green et al. [13] employed comprehensive chromatographic and spectroscopic methods to isolate and characterize terpenoids and flavonoids. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) are used extensively for compound identification, facilitating a nuanced analysis of each compound's bioactivity. Zhou et al. employed robust analytical techniques for measuring phenolic and flavonoid content, with validation to ensure reliability and reproducibility [14].

In their 2021 study, Wu et al. investigated the antibacterial potential of root extracts using spectroscopic techniques. The spectroscopic methods, including UV-Vis, FTIR, and NMR, were employed to characterize the composition of the root extracts. The spectroscopic analysis revealed a range of bioactive compounds in the root extracts, such as polyphenols, flavonoids, and alkaloids. The study highlighted that these compounds, in synergy, provided

broad-spectrum antibacterial effects. Notably, the root extracts demonstrated effectiveness against both Gram-positive and Gram-negative bacteria, which broadens their potential applicability [15].

To the best of our knowledge, there has not been any comparison, in a single publication, between the root and root bark of *Syzygium samarangense*. Therefore, in keeping with our research on *Syzygium samarangense* plant organs, this study aims to ascertain the phytoconstituents, total phenolic content, total flavonoid content, and biological activity of the root and root bark extracts of *Syzygium samarangense* by employing different solvents (aqueous, ethanolic, and dichloromethane) having varying polarities. This research builds on the findings of our recent work [16].

2. Materials and Methods

2.1. Collection and authentication of plant.

The *Syzygium samarangense* plant was harvested fresh from a backyard in Sunyani, Bono Region, Ghana. The plant's roots and root bark were collected. At the Department of Herbal Medicine laboratory of the Faculty of Pharmacy and Pharmaceutical Sciences at Kwame Nkrumah University of Science and Technology in Kumasi, Ghana, the specimens bearing the identification number KNUST/HM1/2024/ROO2 were identified. The samples were then placed in the Herbarium unit of the Herbal Medicine department at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, which is part of the Faculty of Pharmacy and Pharmaceutical Sciences. All of the chemicals used were purchased from Sigma Aldrich in the United States and were of the analytical grade with the highest purity possible (<99.5%).

2.2. Preparation of extracts for phytochemical screening.

For easier air drying, the fresh root and root bark of *Syzygium samarangense* were cut into smaller pieces and cleaned under running water, and then rinsed with distilled water. Using an electric blending machine (Ceado SRL blender, serial No. HIE153001), the dried samples were ground independently. Before extraction, the powdered samples were separated into portions and sealed in appropriately labelled containers.

The extraction of the phytochemicals followed the same methodology that was used in our most recent one [16]. The subsequent procedure is employed to prepare the extracts:

Three distinct round-bottom flasks were filled with about 20 g of the powdered root sample. Then, 200 mL of water, ethanol, and dichloromethane were added to each flask individually. After refluxing for a full day, the mixtures were set aside for a short while. The final mixture was filtered through 125mm Ø Whatman filter paper in each flask, and each solvent was evaporated to dryness using a rotary evaporator (Stuart rotary evaporator, CAT No. RE400/MS) at 40°C and reduced pressure. Before analysis, the dry extracts were kept in a refrigerator at 4°C. The root bark was subjected to this process. For each extract, the recovery % was computed.

2.3. Screening for the phytochemicals in the extracts.

Through minor modifications, we adopted the procedure described by some researchers [17-19] to determine the presence or absence of specific phytochemicals through phytochemical screening of the extracts.

2.4. Quantitative analyses of the total phenolic content and total flavonoid content.

Total phenolic and total flavonoid contents were ascertained by the Folin-Ciocalteu and aluminium chloride methods, respectively. Aqueous (Aqua), ethanolic (EtOH), and dichloromethane (DCM) extracts of stems and stem bark were assayed for total phenolic content using a slightly modified version of the protocol described by Johari and Khong [20]. Ascorbic acid was used as the reference standard in this investigation [21]. With minimal modifications, the conditions outlined by Pełkal and Pyrzyńska [22] were used to investigate the total flavonoid content. The Supplementary material contains the process and outcomes.

2.5. Characterization of the extracts using UV-Visible and FT-IR spectroscopic analyses.

In this investigation, the protocols described by Bashyam et al. [23] were employed with some variation as described in the Supplementary material.

2.6. Antimicrobial effects of *Syzygium samarangense* root and root bark.

Five clinically relevant bacterial strains (W.H.O. priority pathogens): *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter cloacae* were retrieved from the Centre for Research in Applied Biology (CeRAB) microbiology laboratory. Fresh McConkey agar and Mannitol salt agar were prepared according to the manufacturer's instructions. A sterile inoculating loop was used to transfer the bacterial strain from the culture media aseptically and inoculate on the solidified McConkey agar and Mannitol salt agar, respectively, and cultured at 37°C for 24 hours. Bacterial isolates were Gram-stained and biochemically tested using API 20E (**Figures S29 and S30**), and the score was used to confirm the bacterial strains obtained.

2.6.1. Antimicrobial activities on the Muller-Hinton agar.

The disc diffusion method was used to determine the antibacterial activity of *Syzygium samarangense* root and root bark extracts using methods described by Satish et al. [24] with slight modification. The *Syzygium samarangense* root and root bark crude were extracted using ethanol, aqueous, and dichloromethane. The crude extracts were tested against WHO priority pathogenic bacterial strains: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae*. A microbiology loop was used to pick one to two colonies of bacterial cells, and suspended them in 5 mL of normal saline and vortexed for 30 seconds to make an overall suspension, which was compared to the McFarland standard of 0.5 turbidity solution, which was used as a reference to adjust the turbidity of the individual bacterial suspension.

A sterile swab stick was dipped into the bacterial suspension. The swab was rotated against the side of the tube using firm pressure to remove excess fluid, but the swab was not dipped wet. The dried surface of the Muller Hinton agar plate was inoculated by streaking the swab over the entire agar surface by rotating the plate at 60°C to ensure an even distribution of

the inoculum. In each of these plates, 3 wells with a diameter of 6 mm were cut using a sterile cork borer, and agar discs were removed. 0.1 mL of the solvents used for the extracts (dichloromethane, ethanol, and distilled water) was tested against the bacterial strains as a negative control and compared to the efficacy.

The wells were filled with a 0.1 mL sample of each extract (10 mg/mL) using a micro-pipette, kept in a fridge at 2°C to 8°C, and allowed to diffuse for 30 minutes. Ciprofloxacin was used as a positive control. The plates were then incubated upright at 37°C for 24 hours. The zone of inhibition around the central zone was measured according to the guidelines set by the Clinical and Laboratory Standards Institute (CLSI).

2.7. Statistical analysis.

Each investigation was performed on three separate occasions. One-way analysis of variance was used to analyze the data, which are presented as mean ± standard deviation (SD). The SPSS statistical software (Version 28, IBM, Armonk, NY, USA) was employed for evaluating the data. Using the Tukey post hoc test, significant differences (P<0.05) between the groups were observed.

3. Results and Discussion

3.1. Percentage recovery.

The percentage recovery, depicted in Tables 1 and 5, was obtained after they had been expressed as the percentages of the initial mass of each extract.

Compared with the EtOH and DCM extracts, the Aqua extracts produced the highest yield in the root (4.62±0.01) and the root bark (4.51±0.06). When EtOH and DCM root and root bark extracts were compared, EtOH extracts produced a higher yield for the root (3.81±0.06) and root bark (3.67±0.01). With respect to the DCM, the yield of phytochemicals in the root (3.40±0.09) and root bark (3.42±0.02) was found to be higher than expected. The trend and results show that water is the most effective extraction solvent, followed by ethanol, and dichloromethane is the least effective. This is likely due to the fact that the majority of the extracted compounds found in *Syzygium samarangense* root and root bark are polar, making them soluble in water, which is more polar than the non-polar dichloromethane solvents that were used. It is well known that the polarity differences in the substances used in the extraction process have a significant impact on how soluble phytochemical compounds are [25-27].

Once more, the solubility of phytochemicals in different polar solvents can be determined by structural variations in these chemicals [28]. Undoubtedly, the three solvents used in this investigation are polarity-wise arranged as follows: water, ethanol, and dichloromethane. According to the percentage recoveries, this change appears to have had a significant impact on the solubility of phytochemicals in these solvents. The solvent with the least polarity, dichloromethane, had the lowest recovery, while the solvent with the greatest polarity, ethanol, had the highest yield. Thus, the current study's findings have validated the role of solvent in solvent extraction and, as a result, the abundance of polar substances in the root and root bark of *Syzygium samarangense*.

Table 1. The recovery percentage of *Syzygium samarangense* root and root bark extracts.

Extraction solvent	Percentage recovery (%mean±SD)	
	Root	Root bark
Aqua	4.62±0.01	4.51±0.06

EtOH	3.81±0.06	3.67±0.01
DCM	3.40±0.09	3.42±0.02

3.2. Screening for the phytochemicals in the extracts.

Prior to conducting a thorough phytochemical and pharmacological analysis of medicinal plants, qualitative phytochemical screening is a crucial step [29,30].

In this investigation, the phytochemicals were extracted using water, ethanol, and dichloromethane. The extracts were identified as dichloromethane (DCM extracts), ethanol (EtOH extracts), and water or aqueous (Aqua extracts). With the exception of cardiac glycosides, a wide range of secondary metabolites were identified through phytochemical screening assays using the different root and root bark extracts of *Syzygium samarangense* that were utilised in this study (Table 2). Figures S1-S16 show the results of the root and root bark extracts.

Every extract of the root and root bark contained alkaloids. Additionally, essential oils were found in nearly every extract, with the exception of the dichloromethane extracts of the root and root bark. With the exception of the root bark dichloromethane extract, flavonoids were present in every extract of the root and root bark. This is a significant discovery because Guo et al. [31] investigated the bioactive potential of flavonoids derived from the callus tissue of *Ampelopsis grossedentata*, a plant traditionally known for its medicinal properties. The authors focus on the anticancer and antibacterial properties of these flavonoids, proposing them as viable candidates for combating both cancer cell proliferation and bacterial infections. The research addresses a critical need for new agents in these fields, given the rise of drug resistance in both cancer cells and bacterial strains. Similar to alkaloids, phenols were found in every extract. The DCM root extract did not contain any saponins, but saponins were found in the EtOH and Aqua extracts of the roots. Nevertheless, neither the EtOH nor the DCM extracts of the root bark contained any saponins. Tannins were also found in nearly all of the extracts, with the exception of the DCM root and root bark extracts. Once more, the root Aqua and DCM extracts contained terpenoids, but the EtOH extracts did not. In conclusion, regarding the root bark extracts, terpenoids were detected in the DCM extracts but not in the EtOH or Aqua extracts. The use of the Aqua, EtOH, and DCM root and root bark extracts for biological assessment was therefore guided by the presence of these phytoconstituents in the extracts.

Table 2. Phytochemicals were examined in *Syzygium samarangense* root and root bark extracts.

Phytochemical examined	Type of extract	Part of the plant is used	
		Root	Root bark
Alkaloids	Aqua	+	+
	EtOH	+	+
	DCM	+	+
Cardiac glycosides	Aqua	-	-
	EtOH	-	-
	DCM	-	-
Essential oils	Aqua	-	+
	EtOH	+	+
	DCM	-	-
Flavonoids	Aqua	+	+
	EtOH	+	+
	DCM	+	-
Phenols	Aqua	+	+
	EtOH	+	+
	DCM	+	+
Saponins	Aqua	+	+
	EtOH	+	-
	DCM	-	-

Phytochemical examined	Type of extract	Part of the plant is used	
		Root	Root bark
Tannins	Aqua	+	+
	EtOH	+	+
	DCM	-	-
Terpenoids	Aqua	+	-
	EtOH	-	-
	DCM	+	+

(+) = Phytochemical detected; (-) = Phytochemical undetected.

3.3. Total phenolic content (TPC) and total flavonoid content (TFC).

The phytochemicals found in the plant suggest that the plant could be a key source of building blocks for the preparation of more modern synthetic drugs. All three extracts of *Syzygium samarangense* root and root bark contained different compounds, including alkaloids, flavonoids, phenols, and tannins, according to an analysis of the phytochemical screening results. The greatest amount of phytochemicals can be extracted from plant organs using this technique. Phenolic compounds are essential plant constituents that contribute to antioxidant activity because they have redox properties. The TPC of different plant organ extracts was measured using the Folin-Ciocalteu reagent as a starting point. Using ascorbic acid as a standard, the TPC was estimated in this study. The results were obtained from the ascorbic acid calibration curve, and the values were expressed in parts per million as ascorbic acid equivalents (AAE) (Tables 3 and 5, Figure S17).

In the current investigation, the TPC in the Aqua extract was found to be 114.93±0.38 ppm, 112.06±0.74 ppm for the EtOH extract, and 63.25±0.38 ppm for the DCM extract. The TPC of the root bark was found to be 130.41±0.12 ppm in the Aqua extract, 143.68±0.62 ppm in the EtOH extract, and 63.25±1.84 ppm in the DCM extract. By fitting the standard on a straight line, it was discovered to be linear in the range of 100 to 180 ppm, producing the equation $y = 0.00325x - 0.00672$.

Table 3. Results of the total phenolic content of the root and root bark of *Syzygium samarangense*.

Extract	Total phenolic content (mean±SD) ppm	
	Root	Root bark
Aqua	114.93±0.38	130.41±0.12
EtOH	112.06±0.74	143.68±0.62
DCM	63.25±0.38	63.25±1.84

To determine TFC, the aluminium chloride method was employed. The technique is based on nitrating quercetin's aromatic ring, which is non-sterically hindered at position 3 or 4. This produces a yellow complex of aluminium that turns red when sodium hydroxide is added [21]. Figure S18 displays the calibration curve that was created with quercetin serving as the standard.

TFC values for aqueous extracts were found to be higher than those of ethanolic and dichloromethane extracts. For example, the TFC values for the root were 46.07±0.31 ppm for the dichloromethane extract, 113.04±0.47 ppm for the ethanolic extract, and 104.01±0.43 ppm for the aqueous extract. TFC values in the root bark were measured and found to be 142.17±0.47 ppm for the aqueous extract, 148.53±0.27 ppm for the ethanolic extract, and 50.68±0.31 ppm for the dichloromethane extract. The aqueous extracts had the highest TFC values in both the root and the root bark, followed by the ethanolic extracts and the dichloromethane extracts, which had the lowest TFC values. This may have occurred because a significant amount of the relatively polar flavonoids were extracted by water, the solvent with the highest polarity [32,33]. Flavonoid heteroside content is higher in plant extracts with low

TPC than aglicone content [28]. Overall, it should be emphasised that the recovery of phytochemicals from plants is influenced by the dielectric constant, the chemical composition of organic solvents, and the chemical properties of plant phytochemicals [29] (Tables 4 and 5).

Table 4. Results of the total flavonoid content of the root and root bark of *Syzygium samarangense*.

Extract	Total flavonoid content (mean±SD) ppm	
	Root	Root bark
Aqua	104.01±0.43	142.17±0.47
EtOH	113.04±0.47	148.53±0.27
DCM	46.07 ±0.31	50.68±0.31

Table 5. Comparison of extract yield, TPC, and TFC of the root and root bark extracts of *Syzygium samarangense*.

Extract	Recovery (mean±SD%)		TPC (mean±SD) ppm		TFC (mean±SD) ppm	
	Root	Root Bark	Root	Root Bark	Root	Root Bark
Aqua	4.62±0.01	4.51±0.06	114.93±0.38	130.41±0.12	104.01±0.43	142.17±0.47
EtOH	3.81±0.06	3.67±0.01	112.06±0.74	143.68±0.62	113.04±0.47	148.53±0.27
DCM	3.40±0.09	3.42±0.02	63.25±0.38	63.25±1.84	46.07 ±0.31	50.68±0.31

3.4. Characterization of the phytochemicals by UV-visible spectroscopy.

At wavelengths between 200 and 800 nm, compounds containing aromatic rings, chromophores, σ -bonds, and lone pairs of electrons were found in extracts from the root and root bark of *Syzygium samarangense*. The spectra of the various extracts are shown in Figure 1 and Figures S19-23.

The Aqua extract of the root exhibited absorption at the following wavelengths: 200, 212, 273, 460, 495, 518, 595, 731, and 779 nm. The EtOH extract of the roots showed absorption at the following wavelengths: 200, 295, 459, 495, 512, 563, 687, 743, and 783 nm; and the DCM extract at the following wavelengths: 200, 227, 311, 464, and 687 nm (Figure 1 and Figures S19 and S20). After analysing each root extract, it was seen that the absorption peaks with the lowest and highest values were 200 and 799 nm, respectively. These absorption bands are caused by phenols, flavonoids, and their derivatives in the extracts [23].

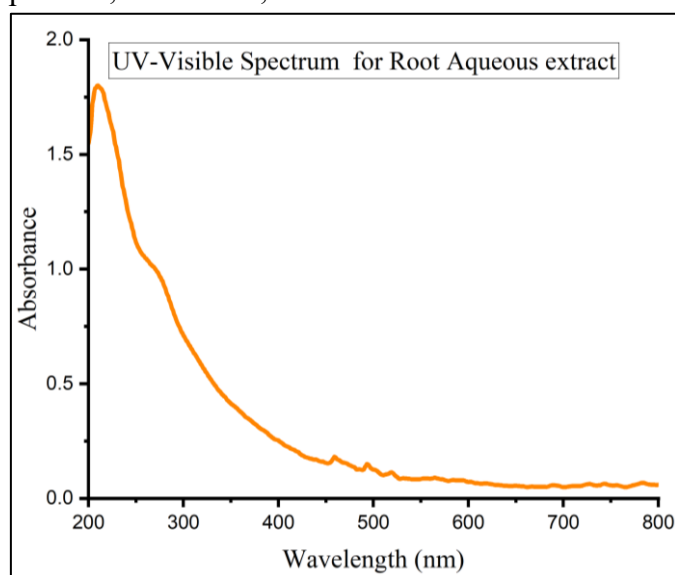


Figure 1. UV-Vis spectrum of the root Aqua extract displaying absorptions and absorption wavelengths indicating the presence of certain bonds.

The absorptions at 200, 208, 246, 276, 460, 494, 522, 722, and 789 nm (Aqua extract), 200, 210, 221, 279, 404, 463, 491, 492, 568, and 775 nm (EtOH extract), and 200, 227, 247, 402, 460, and 692 nm (DCM extract) were recorded in the root bark extracts (Figure 1 and

Figures S21-23). In conclusion, the root bark analysis revealed that the absorption peaks occurred between 200 and 789 nm. The extracts' flavonoid, phenolic, and derivative content is what causes these absorption bands [23]. In a nutshell, the absorption bands observed in the root and the root bark extracts (Aqua, EtOH, and DCM) of the plant indicate the presence of phytochemicals such as tannins, flavonoids, phenols, alkaloids, and their derivatives [23].

3.5. Characterization of the phytochemicals by FT-IR spectroscopy.

The Fourier-transform spectra of the different *Syzygium samarangense* root and root bark extracts (Aqua, EtOH, and DCM) are displayed in Figure 2 and Figures S24-28. The -OH group (3294 cm^{-1}) is visible in the spectrum of the Aqua extract from the roots of *Syzygium samarangense*. The C-N stretching occurs at 2063 cm^{-1} . The carbonyl or ketonic group (C=O) can be seen at 1628 cm^{-1} , which is one of the common characteristics of flavonoids. The -CH₃ is visible at 1394 cm^{-1} , while the C-O is indicated at 1073 cm^{-1} (Figure 2).

Regarding the root EtOH extract (Figure S24), the -OH group is depicted at 3306 cm^{-1} . At 2973 cm^{-1} , the aliphatic -CH stretch is prominent. The C-N stretching is observed at 2131 cm^{-1} . The carbonyl is indicated by the C=O stretching peak at 1634 cm^{-1} . The presence of the -CH₃ group is shown at 1379 cm^{-1} . Absorptions at 1046 and 875 cm^{-1} reveal the C-N and =CH bonds, respectively. Figure S25 depicts the spectrum of *Syzygium samarangense* DCM root extract. The peaks at 2918 cm^{-1} and 2852 cm^{-1} indicate the presence of -CH. The a ketonic C=O group is seen at 1726 cm^{-1} , 1465 cm^{-1} shows Ar-C=C, 1373 cm^{-1} indicates -CH₃, whilst 1021 cm^{-1} and 729 cm^{-1} illustrate the presence of the C-N and C-Br bonds, respectively. In summary, the O-H, -CH, C≡N, C=O, Ar-C=C, -CH₃, C-O, C-N, =C-H, and C-Br groups are the functional groups found in *Syzygium samarangense* root extracts [33].

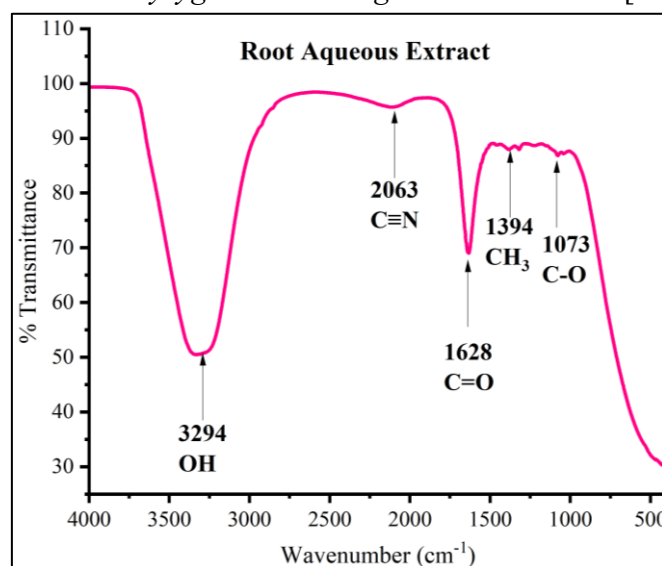


Figure 2. FT-IR spectroscopy of *Syzygium samarangense* root aqua extract reveals absorption and associated functional groups.

The Aqua extract of the root bark of *Syzygium samarangense* (Figure S26) exhibits absorption at 3279 cm^{-1} , suggesting the presence of the -OH group. The C≡N group is at 2089 cm^{-1} . At 1645 cm^{-1} , the C=O carbonyl group is located. The C-O and CH₃ groups are found at 1124 and 1369 cm^{-1} , respectively. The presence of the -OH group is indicated at 3332 cm^{-1} in the root bark EtOH extract (Figure S27). At 2982 cm^{-1} , there is an aliphatic -CH stretching absorption. The C≡N is found at 2089 cm^{-1} . The presence of a C=O group is indicated at 1664 cm^{-1} . The hydrocarbon -CH₃ chain is found at 1379 cm^{-1} . An amine-type C-N stretching peak

arises at 1044 cm⁻¹. A =CH group is displayed at 878 cm⁻¹. The *Syzygium samarangense* DCM root bark extract spectrum (Figure S28) verifies the existence of -CH at 2920 and 2851 cm⁻¹. Absorption at 1736 cm⁻¹ indicates the presence of a ketonic C=O group. The absorption at 1472 cm⁻¹ indicates the presence of an aromatic Ar-C=C group. The presence of C-O and C-N groups is indicated by absorptions at 1188 and 1031 cm⁻¹, respectively, whereas the presence of C-Br is shown at 720 cm⁻¹. Accordingly, O-H, -CH, C=O, Ar-C=C, CH₃, C-O, C-N, C≡N, and C-Br groups are present in the Aqua, EtOH, and DCM extracts of the root bark of *Syzygium samarangense* [34].

In summary, the different functional groups sighted in different extracts manifested the biochemical profile of the root and the root bark extracts of *Syzygium samarangense*, which could be responsible for their various antimicrobial effects.

3.6. Antimicrobial effect of the root and root bark of *Syzygium samarangense*.

As the search for alternative medicine to combat infections caused by antibiotic-resistant bacteria has gained significant momentum, Song et al. provided evidence supporting flavonoid compounds as potential antibacterial agents against multidrug-resistant pathogens [35]. A lot of research has shown the neuroprotective properties of essential oils extracted from several plants, such as *Ocimum gratissimum*, both in vivo and in vitro [36].

In our present study, we have investigated the antimicrobial potential of the Aqua, EtOH, and DCM extracts of *Syzygium samarangense* root and root bark against some clinically relevant bacteria.

The mean and standard deviation of the antimicrobial potential of Aqua, EtOH, and DCM extracts of *Syzygium samarangense* root and root bark were tested on clinically relevant bacteria. The results are presented in Tables 6 and 7.

Table 6 presents the estimated means of the zone of inhibition (mm) based on the organisms and root bark extracts. The data suggest that Ciprofloxacin (Positive Control) was generally the most potent, followed by the EtOH extracts, and then the Aqua extracts. The DCM extracts exhibited the lowest effectiveness across all organisms. Interestingly, the EtOH extracts demonstrated higher efficacy against *Enterobacter cloacae* than Ciprofloxacin.

The findings of this study revealed that the EtOH extracts had greater effects on all the tested bacterial strains except against *Escherichia coli* (10.67±2.1), which was susceptible to the Aqua extracts (11.33±4.0) as compared to the EtOH extract. *Staphylococcus aureus* exhibited significantly larger zones of inhibition (Aqua Extract = 14.0±2.0; EtOH extract = 18.67±3.0; DCM Extract = 9.33±1.5) compared to *Pseudomonas aeruginosa* (Aqua Extract = 12.33±1.5; EtOH extract = 14±2.65; DCM Extract = 7.67±0.58). Additionally, all the extracts showed a greater zone of inhibition against *Klebsiella pneumoniae* than against *Escherichia coli*. Finally, *Enterobacter cloacae* generally exhibited smaller zones of inhibition. Surprisingly, the EtOH extracts showed a greater zone of inhibition for *Enterobacter cloacae* compared to *Pseudomonas aeruginosa* and *Escherichia coli*.

Table 6. The mean inhibitory activities of the crude extracts of *Syzygium samarangense* root bark against different clinically relevant bacteria strains.

Bacterial strains	Zone of Inhibition (mm)			Ciprofloxacin (10 µg/disc)
	Aqua extract	DCM extract	EtOH extract	
<i>Escherichia coli</i>	11.33±4.0	7.0±1.0	10.67±2.1	24.0±3.0
<i>Klebsiella pneumoniae</i>	14.67±1.5	9.0±1.0	17.33±1.5	20.33±1.5
<i>Staphylococcus aureus</i>	14.0±2.0	9.33±1.5	18.67±3.0	20±2
<i>Enterobacter cloacae</i>	9±1.0	6.67±0.6	16.7±3.1	12±5.2

Bacterial strains	Zone of Inhibition (mm)			Ciprofloxacin (10 µg/disc)
	Aqua extract	DCM extract	EtOH extract	
<i>Pseudomonas aeruginosa</i>	12.33±1.5	7.67±0.58	14±2.65	19.3±4.0

Our findings on the root extracts (Table 7) showed that EtOH extracts have a higher inhibition (16.33±0.6) against *Klebsiella pneumoniae*, followed by *Escherichia coli* (12.33±1.5 mm). The inhibition of the EtOH extracts against *Pseudomonas aeruginosa* was the least (10.67±1.16 mm). Additionally, the EtOH extracts exhibited higher inhibition against *Staphylococcus aureus* (14.33±0.6 mm) and *Enterobacter cloacae* (14.0±2.0 mm). The DCM extracts showed a lower zone of inhibition towards all the bacterial strains. Comparing the results of the root extracts to the Ciprofloxacin (10 µg/disc) against the bacterial strains, the EtOH (14.33±2.1 mm) and the Aqua (14.0±2.0 mm) extracts exhibited a greater efficacy towards *Enterobacter cloacae* than that of Ciprofloxacin (13.33±5.1 mm).

Table 7. The mean inhibitory activities of the crude extracts of *Syzygium samarangense* root against different clinically relevant bacteria strains.

Bacterial Strains	Zone of Inhibition (mm)			Ciprofloxacin (10 µg/disc)
	Aqua extract	DCM extract	EtOH extract	
<i>Escherichia coli</i>	10.67±2.1	6.0±0.0	12.33±1.5	23.33±3.0
<i>Klebsiella pneumoniae</i>	12.33±1.5	7.33±0.6	16.33±0.6	20.33±1.5
<i>Staphylococcus aureus</i>	14.33±0.6	8.0±1.0	13.33±3.5	20.0±1.0
<i>Enterobacter cloacae</i>	14.0±2.0	9.0±2.0	14.33±2.1	13.33±5.1
<i>Pseudomonas aeruginosa</i>	11.26±2.6	7.67±1.16	10.67±1.16	18.33±5.5

Summing up, comparing the results of the root and the root bark extracts (Aqua, EtOH, and DCM extracts) of *Syzygium samarangense*, in terms of efficacy against the bacterial strains, the EtOH extract of the root exhibited a significant inhibition of 12.33 ± 1.5 mm against *E. coli*, while the EtOH extract of the root bark showed an inhibition of 10.67 ± 2.1 mm. Additionally, *Klebsiella pneumoniae* was observed to be susceptible to both the Aqua and EtOH extracts, with inhibition zones of 14.67 ± 1.5 mm and 17.33 ± 1.5 mm, respectively. In comparison, the root extracts had minimum inhibition zones of 12.33 ± 1.5 mm and 16.33 ± 0.6 mm, for Aqua and EtOH, respectively. Moreover, both the Aqua and EtOH extracts of the root were found to be more effective against *Enterobacter cloacae*, with inhibition zones of 14.0 ± 2.0 mm and 14.33 ± 2.1 mm, respectively. This organism showed less sensitivity to the root bark extract. Furthermore, when the extracts were tested against *Pseudomonas aeruginosa*, the EtOH extract of the root bark demonstrated a promising inhibition of 14.0 ± 2.65 mm, while the EtOH root extracts displayed an inhibition of only 10.67 ± 1.16 mm. Finally, the DCM extracts (Aqua, EtOH, and DCM) of the root and root bark of *Syzygium samarangense* were found to be less sensitive against all the bacterial pathogens.

4. Conclusions

In conclusion, and based on the study's findings, the majority of the extracts contained seven of the eight phytochemicals. The total amount of phenolic and flavonoid compounds in the extracts suggests that the root and root bark of *Syzygium samarangense* may be possible sources of a natural antimicrobial agent. Once more, the results of this investigation showed that phytochemicals, including alkaloids, essential oils, flavonoids, phenols, saponins, tannins, and terpenoids—which may function singly or in concert—may be responsible for the medicinal potential of *Syzygium samarangense* root and root bark.

The antimicrobial activities of *Syzygium samarangense* root and root bark extracts demonstrate significant potential as alternative treatments against clinically relevant bacteria

such as *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The EtOH and Aqua extracts show promise in inhibiting bacterial growth, which could contribute to the development of new plant-based antimicrobial agents.

In a nutshell, this research has been able to investigate and report the presence of phytochemicals in different extracts of root and root bark of *Syzygium samarangense*, utilising various solvents with varying polarities in order to ascertain the synergic effects of these extracts with respect to their antimicrobial effects. Having achieved this, our future investigations will focus on the isolation and characterization of these bioactive compounds in these extracts by using advanced hyphenated techniques such as LC-MS, HPLC-MS, GC-MS, etc. Any findings will be reported appropriately.

Author Contributions

Conceptualization, I.Y. and S.F.G.; methodology, B.A.O. and I.W.; software, B.A.O. and I.W.; validation, I.Y. and S.F.G.; formal analysis, B.A.O. and I.W.; investigation, B.A.O. and I.W.; resources, I.Y. and S.F.G.; data curation, I.Y., B.A.O. and I.W.; writing—original draft preparation, I.Y. and I.W.; writing—review and editing, I.Y.; visualization, I.Y.; supervision, I.Y.; project administration, I.Y.; funding acquisition, N/A. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

No funding has been received for this investigation.

Acknowledgments

The authors wish to say thank you to the Department of Chemical Sciences and the Department of Biological Sciences, School of Sciences of the University of Energy and Natural Resources.

Conflicts of Interest

There is no conflict of interest, according to the authors.

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Supplementary Materials

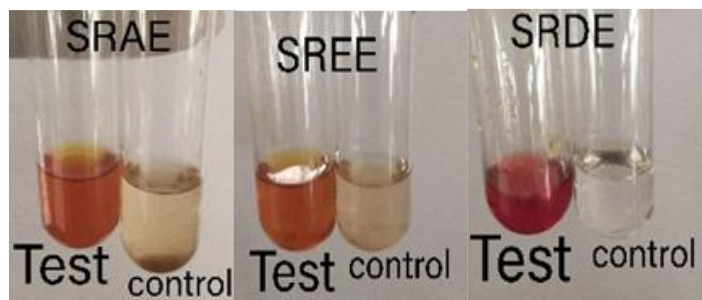


Figure S1. Results of the test for Alkaloids in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

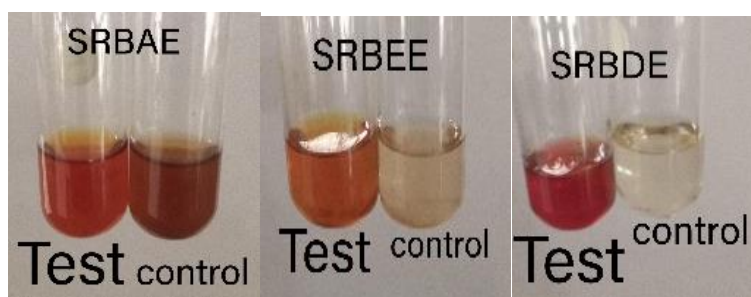


Figure S2. Results of the test for Alkaloids in the Root Bark Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

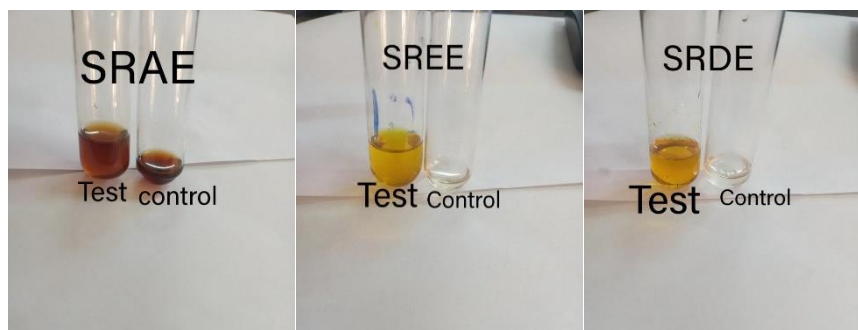


Figure S3. Results of the test for Cardiac Glycosides in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

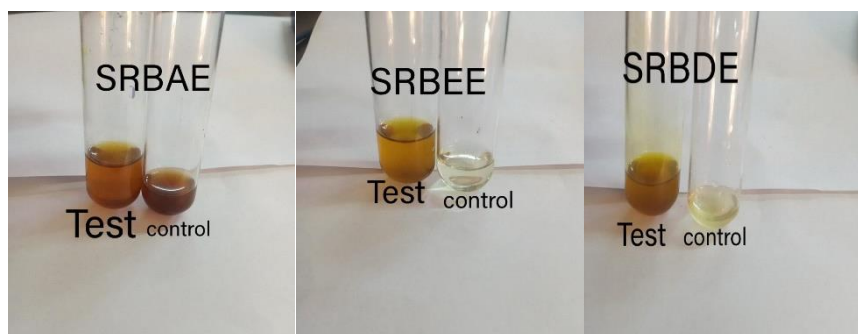


Figure S4. Results of the test for Cardiac Glycosides in the Root Bark Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

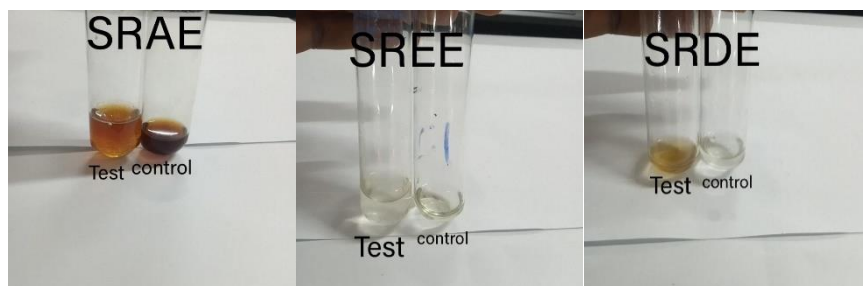


Figure S5. Results of the test for Essential Oils in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

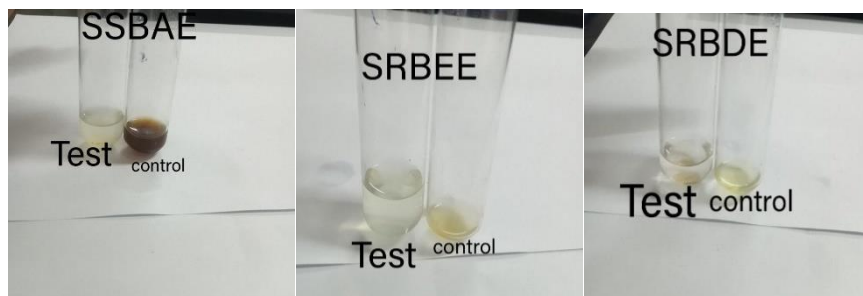


Figure S6. Results of the test for Essential Oils in the Root Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

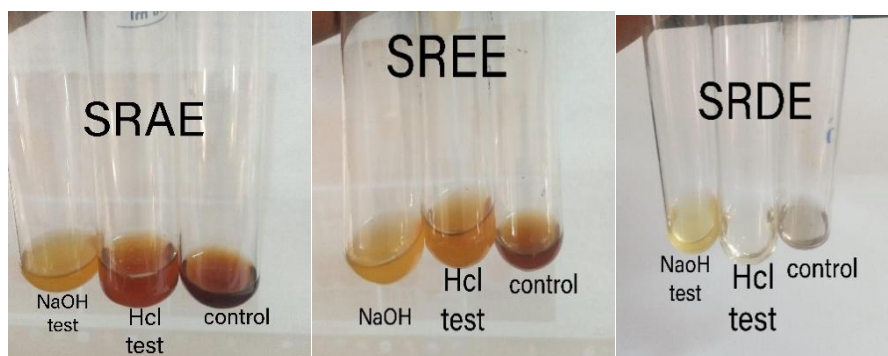


Figure S7. Results of the test for Flavonoids in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

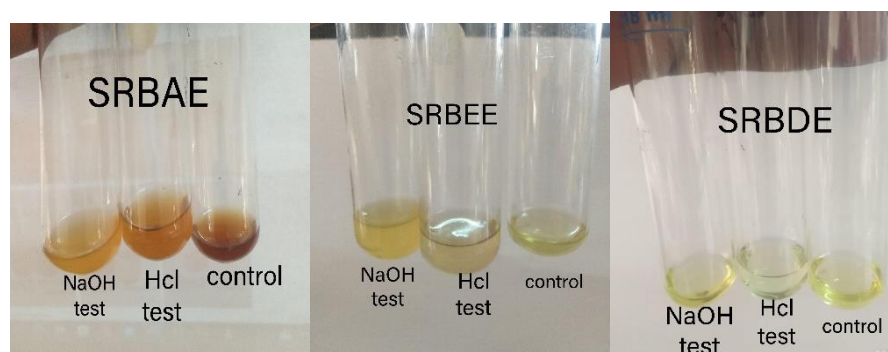


Figure S8. Results of the test for Flavonoids in the Root Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

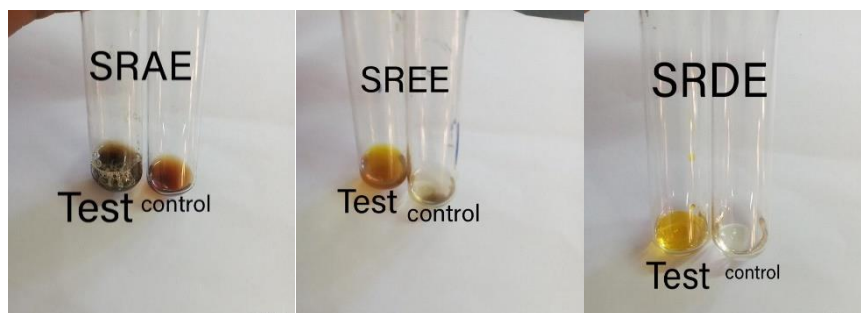


Figure S9. Results of the test for Phenols in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

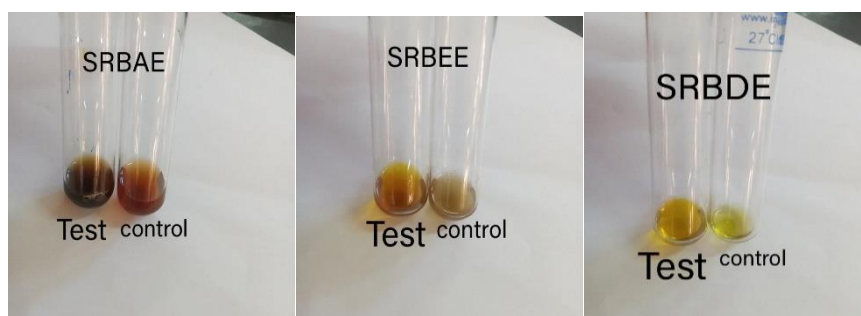


Figure S10. Results of the test for Phenols in the Root Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

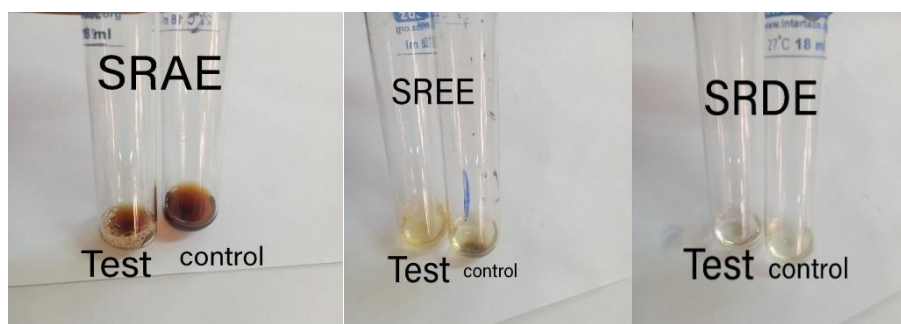


Figure S11. Results of the test for Saponins in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

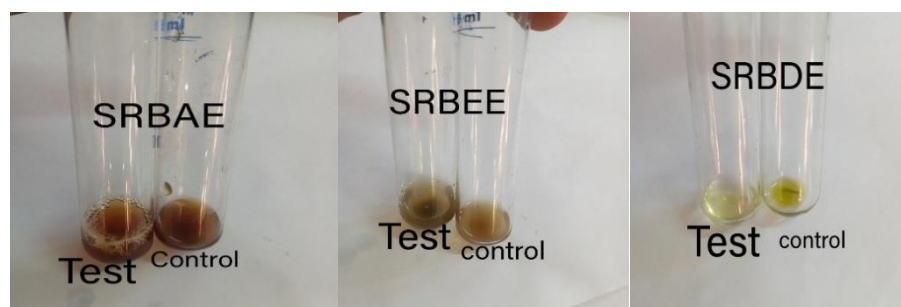


Figure S12. Results of the test for Saponins in the Root Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

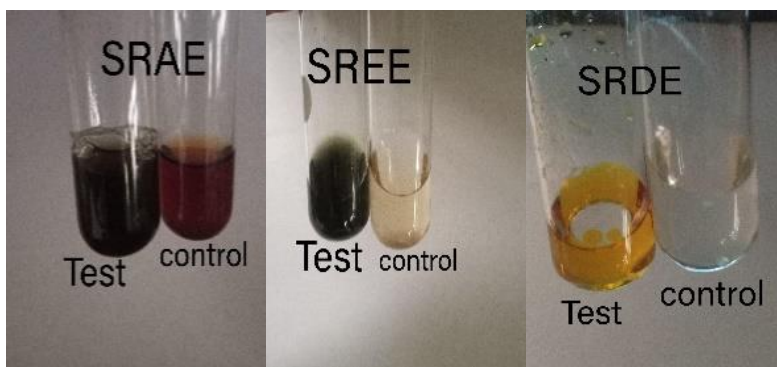


Figure S13. Results of the test for Tannins in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

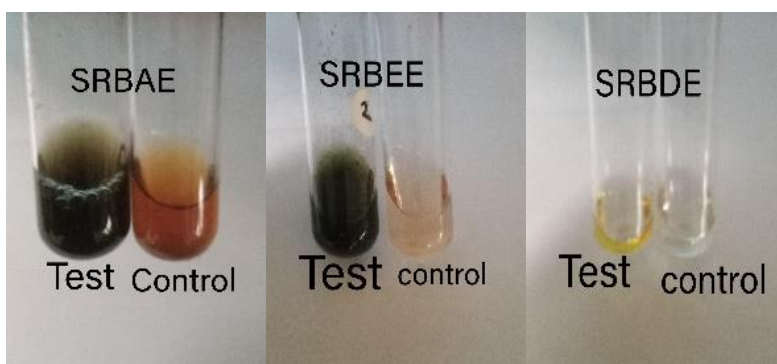


Figure S14. Results of the test for Tannins in the Root Bark Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

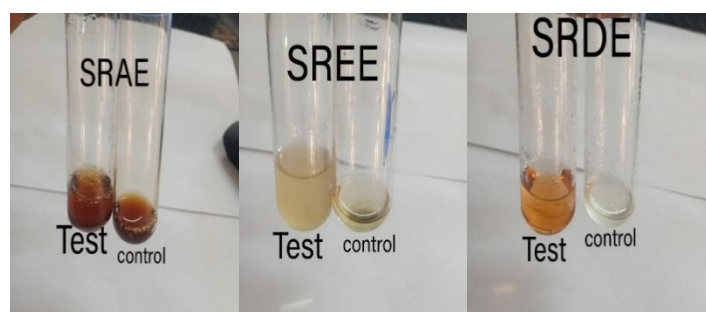


Figure S15. Results of the test for Terpenoids in the Root Extract (SRAE = *Syzygium samarangense* Root Aqueous Extract; SREE = *Syzygium samarangense* Root Ethanolic Extract; SRDE = *Syzygium samarangense* Root Dichloromethane Extract).

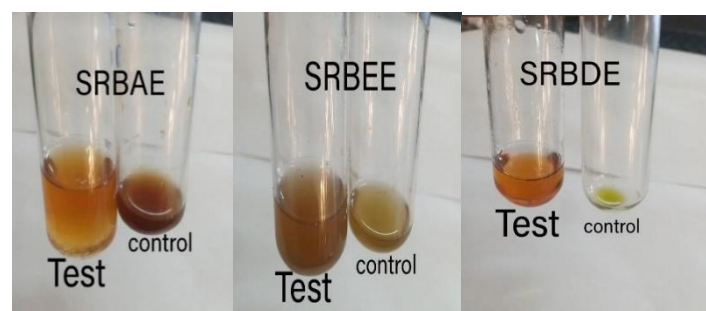


Figure S16. Results of the test for Terpenoids in the Root Bark Extract (SRBAE = *Syzygium samarangense* Root Bark Aqueous Extract; SRBEE = *Syzygium samarangense* Root Bark Ethanolic Extract; SRBDE = *Syzygium samarangense* Root Bark Dichloromethane Extract).

Table S1. Plotted Values for TPC Investigation.

Concentration (ppm)	Absorbance
0	0
100	0.383
120	0.469
140	0.561
160	0.651
180	0.731

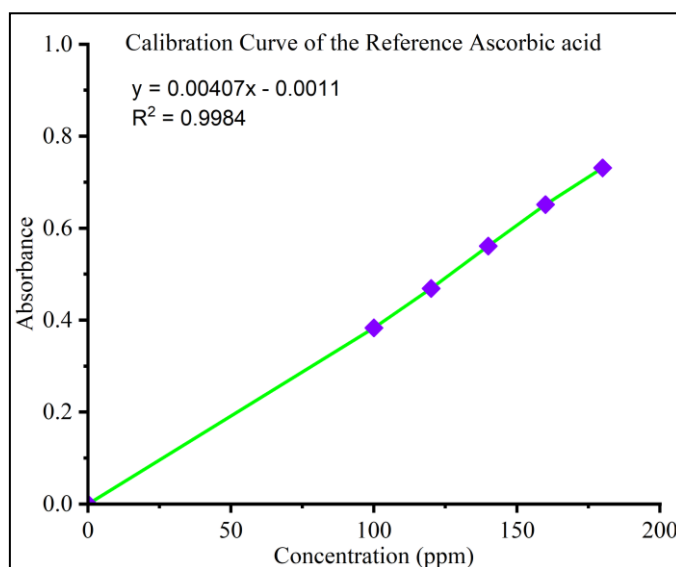


Figure S17. Calibration Curve for the determination of Total Phenolic Content using Ascorbic acid as Reference.

Table S2. Plotted Values for Total Flavonoid Content Determination

Concentration (ppm)	Absorbance
0	0
100	0.308
120	0.383
140	0.441
160	0.515
180	0.591

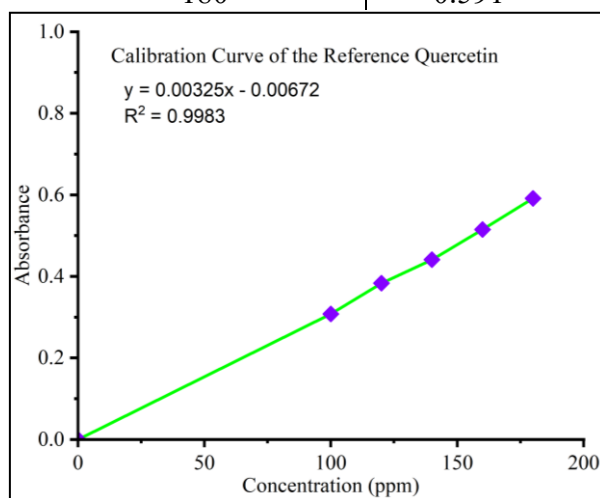


Figure S18. Calibration Curve for the determination of Total Flavonoid Content using Quercetin as Reference.

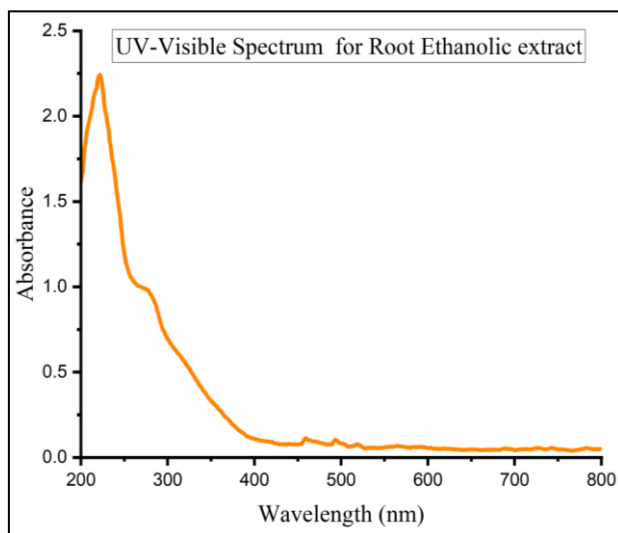


Figure S19. UV-Vis spectrum of the root ethanolic extract displaying absorptions and absorption wavelengths indicating the presence of certain bonds.

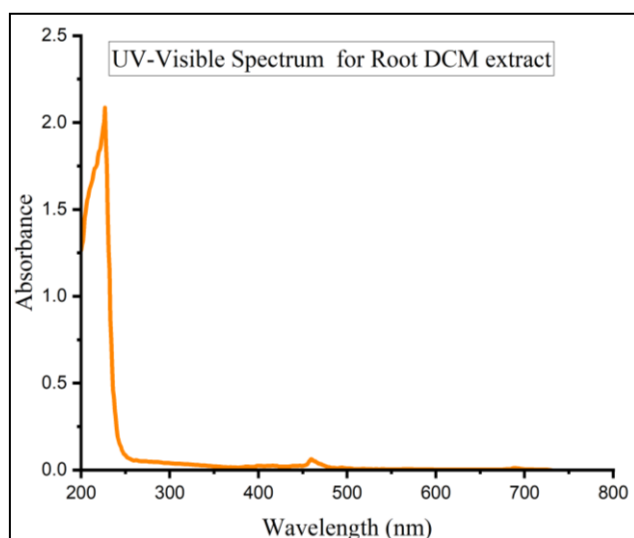


Figure S20. UV-Vis spectrum of the root dichloromethane extract displaying absorptions and absorption wavelengths indicating the presence of certain bonds.

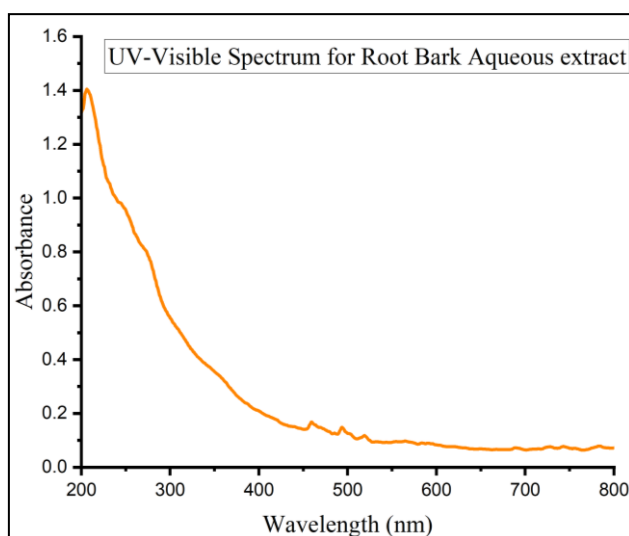


Figure S21. UV-Vis spectrum of the root bark aqueous extract displaying absorptions and absorption wavelengths indicating the presence of certain bonds.

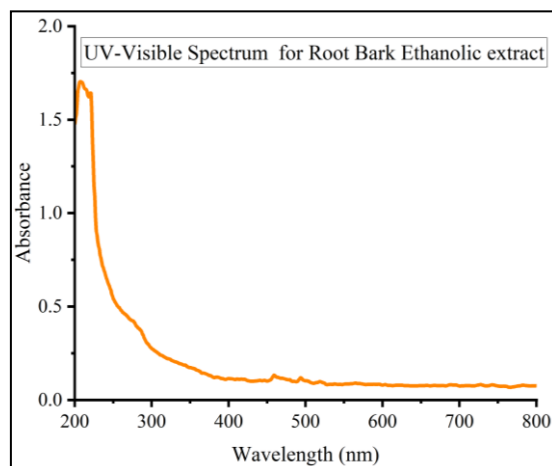


Figure S22. UV-Vis spectrum of the root bark ethanolic extract displaying absorptions and absorption wavelengths indicating the presence of certain bonds.

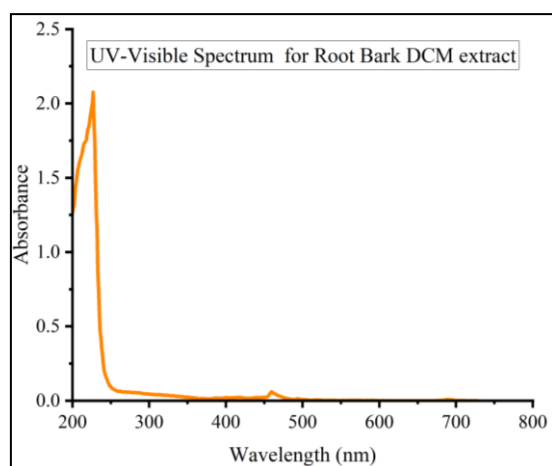


Figure S23. UV-Vis spectrum of the root bark dichloromethane extract displaying absorptions and absorption wavelengths indicating the presence of certain bonds.

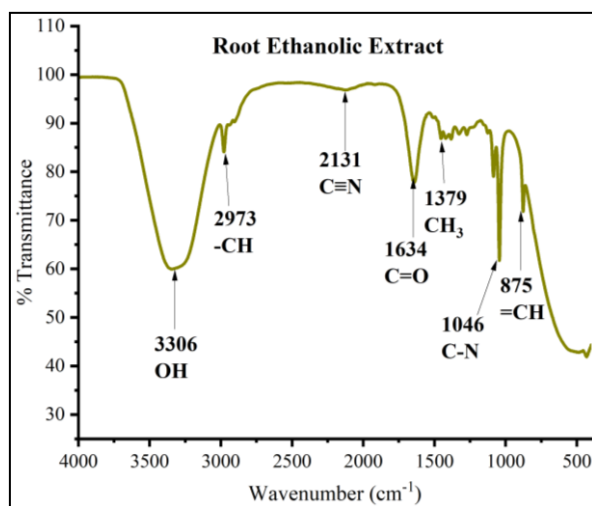


Figure S24. FT-IR infrared spectroscopy of *Syzygium samarangense* root ethanolic extract reveals absorption and associated functional group.

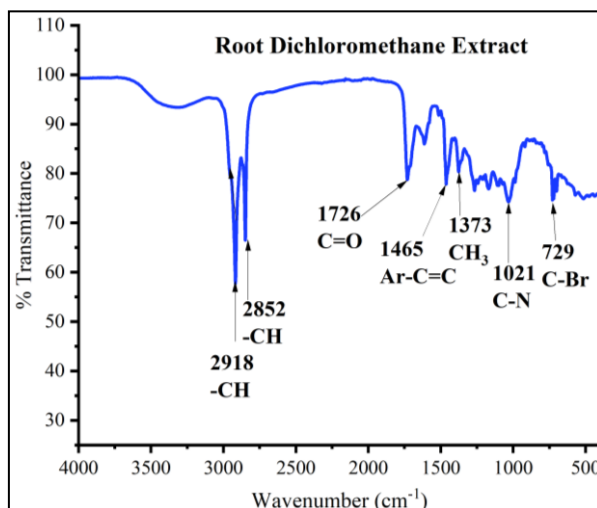


Figure S25. FT-IR spectroscopy of *Syzygium samarangense* root dichloromethane extract reveals absorption and associated functional group.

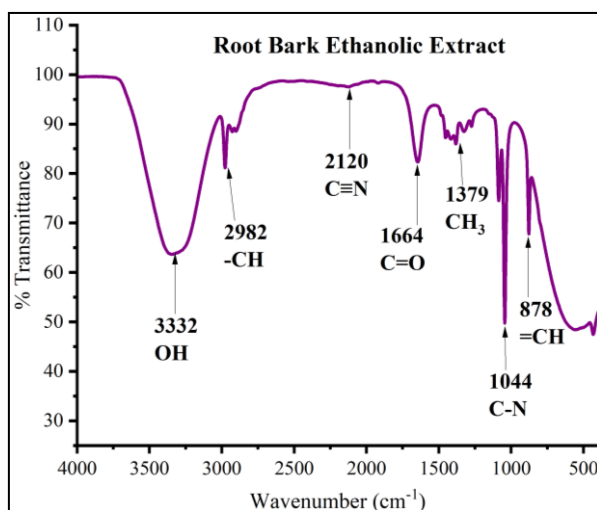


Figure S27. FT-IR spectroscopy of *Syzygium samarangense* root bark ethanolic extract reveals absorption and associated functional group.

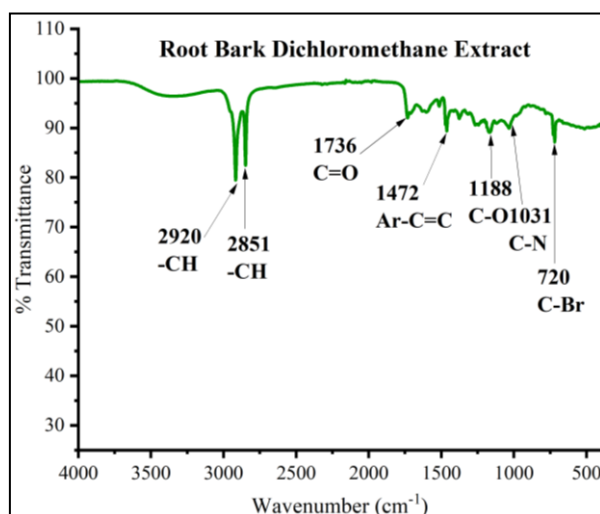


Figure S28. FT-IR spectroscopy of *Syzygium samarangense* root bark dichloromethane extract reveals absorption and associated functional group.

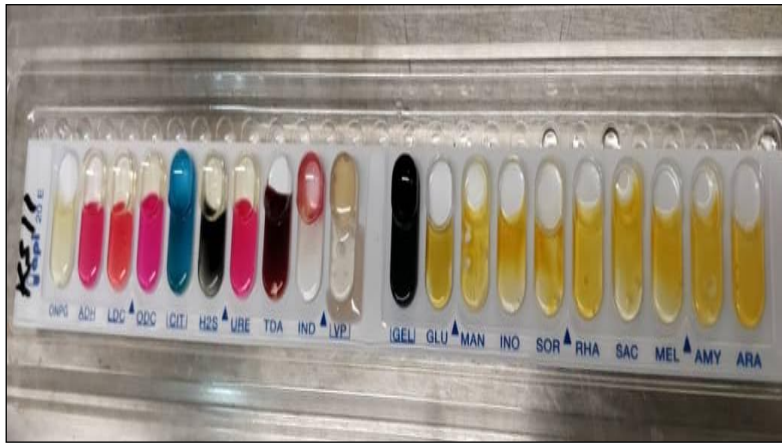


Figure S29. Biochemical test for bacterial identification.



Figure S30. API 20 E.

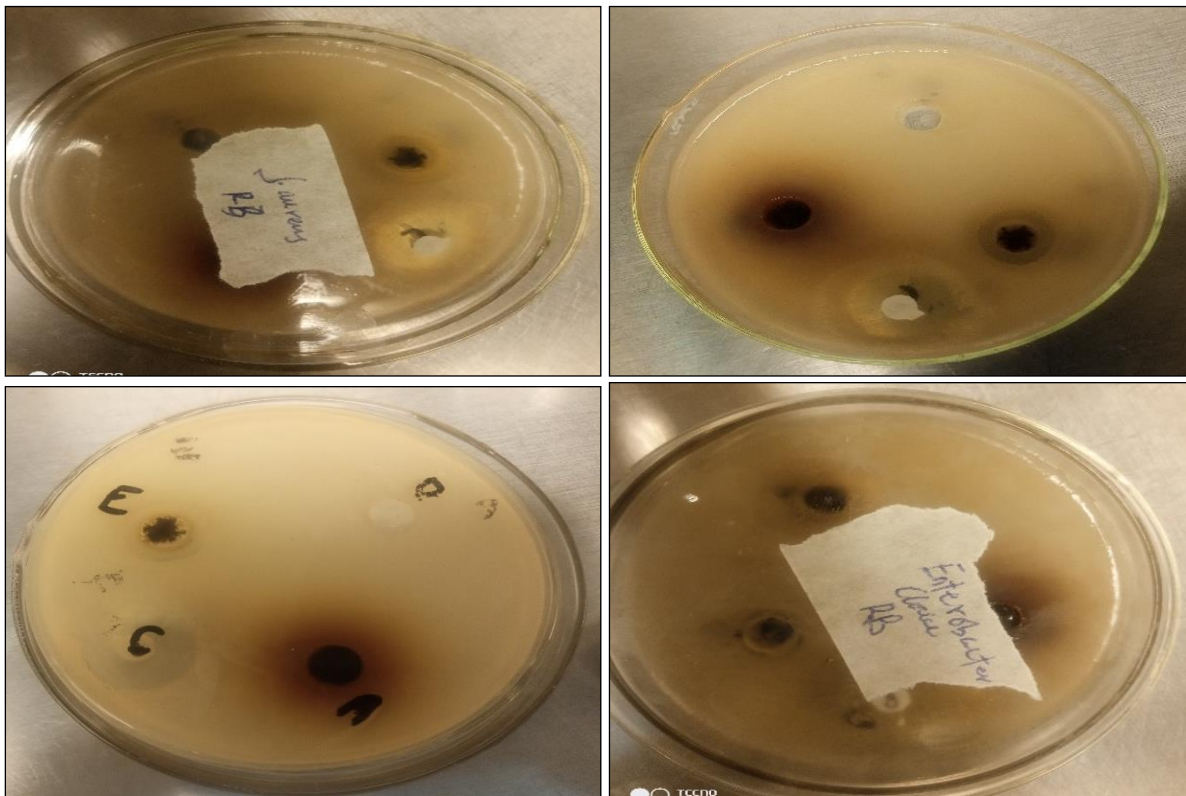


Figure S31. Plates showing antimicrobial activities of *Syzygium samarangense* root and root bark extracts.

Table S3. Morphological Characteristics of the Bacterial strains.

Bacterial Strains	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterobacter cloacae</i>
Cell morphology (cell shape)	Coccus	Rod	Rod	Rod	Rod
Gram reaction	Positive	Negative	Negative	Negative	Negative

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