Int.J.Curr.Microbiol.App.Sci (2026) *15*(1): 194-205

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 1 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1501.024

Comparison of antimicrobial effect and phytochemical profile of *Phyllanthus niruri* and *Syzygium aromaticum* extracts against some selected bacteria

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Keywords

Phytochemicals, Pathogenic Bacteria, Antibiotics, Extracts

Article Info

Received:
18 October 2025
Accepted:
15 December 2025
Available Online:
10 January 2026

A B S T R A C T

The traditional medicinal plants *Phyllanthus niruri* and *Syzygium aromaticum* have numerous phytochemicals that make them valuable in medicine such as alkaloids, flavonoid, saponins, phenols and steroids. This paper examined the antimicrobial activity of their solvent extracts by confirming ethnomedicinal suitability and determining the potential as complementary antimicrobials. The leaves and fruits of *P. niruri* were dried, then powdered and extracted with ethanol, distilled water and boiled water. Agar well diffusion was used to test the antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* with cefuroxime and ciprofloxacin serving as controls. Inhibition zones were recorded after incubating the tissues at 37^0C after 24 hours. Phytochemical screening reported all the target bioactive compounds in the two species. It was revealed that, ethanolic extracts were more effective in antibacterial activities than distilled water extracts and boiled water extracts, which were relatively less effective in this regard with some being even equal to or greater than the standard antibiotics. It was established that *Syzygium aromaticum* was more effective in antibacterial activities when compared to distilled water extracts and boiled water extracts which were relatively weak. On the whole, *S. aureus* showed the greatest susceptibility, then *E. coli* and moderate sensitivity of *S. typhi* was demonstrated. These results support the significant antibacterial activities of the two plants and especially the broad-spectrum activity of *S. aromaticum*, which will justify the use and eventual therapeutic utility of both.

Introduction

The use of antibiotics has been and continues to be one of the most influential medical discoveries in the 20th and 21st centuries with the applications drastically cutting the world morbidity and mortality related to bacterial infections (11). Antimicrobials since the discovery of penicillin have been core in the development of public-health. However, irrespective of significant progress in diagnostics, treatment, and surveillance, infectious diseases still have a disproportionate impact on the low- and middle-income countries (LMICs) (17). Bacterial infections are also major causes of death in Sub-Saharan Africa and South Asia according to global disease-burden assessments, as they demonstrate endemic inequities in how people can access, afford, and obtain effective antibiotics (25, 27). They are intensified by the ineffectiveness of pharmaceutical regulatory frameworks, the inability to supply the antimicrobials with adequate quality assurance due to economic constraints (26, 30, 35). *Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus* are some of the high priority pathogens within LMICs. *Escherichia coli* is a primary cause of UTI, neonatal sepsis, and diarrheal diseases (22); *Salmonellosis typhi*, causing typhoid fever, is endemic and causes a considerable amount of morbidity and mortality where water and sanitary treatments are insufficient (25); and *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), causes soft tissue disease, bacteremia, and endocarditis and is increasingly showing multidrug-resistant phenomena (27). The growing problem of treating infections by these organisms highlights the immediate necessity of having alternative sources of antimicrobials around the world. As a result, there has been an interest in medicinal plants by science. As defined by the World Health Organization, as many as 70-80 percent of the population in the developing countries use herbal forms of medicine as the initial line of treating problems as such treatment is available, not expensive, and is culturally acceptable (31, 35). This has been driven by the fact that synthetic antibiotics have adverse reactions some of which are gastrointestinal injury, hepatotoxicity, allergies, and organ damage which are major causes of hospitalization and even mortality caused by the drug (10). Herbal preparations are widely considered softer and are becoming a part of holistic and integrative practice of health (33). Medicinal plants have great potential in drug discovery since they are abundant sources of secondary metabolites which include flavonoids, phenolics, tannin, terpenoids, lignans and alkaloids (3, 12, 23). These compounds show a wide array of pharmacological activity, and antimicrobial, antioxidant, anti-inflammatory, immunomodulatory and anticancer activity (5,29). A variety of common antibiotics and other treatment agents are found in the botanical origin, and natural-product scaffolds constitute a substantial part of current medicines (3, 24). Multidrug resistance is increasing, and the number of new antibiotics being developed is dwindling so the search to find new plant-based antimicrobial compounds is becoming more urgent (7, 23). One of such medicinal plants that has extensive therapeutic value is *Phyllanthus niruri*. *P. niruri* is also widespread in the tropical world such as Ghana, Southeast Asia, and South America, and long its usage has been in the treatment of kidney stones, liver diseases, infection and inflammatory diseases (4, 13,20). The phytochemical research has revealed that it comprises alkaloids, flavonoids, ellagitannin, phenolic acids as well as lignans--all these are linked to antimicrobial, hepatoprotective, antioxidant, antihyperglycemic and antiviral properties (9, 18,). Its extracts have been found to have activity against *E. coli, S. typhi* and *S. aureus* in previous research studies (13, 18). In the same way, *Syzygium aromaticum* (clove) is a commonly used medical and cooking herb used in therapeutic uses around the globe. Its volatile oil which is rich in phenolic compounds like the phenol's eugenol has got the strong antimicrobial, analgesic, anti-inflammatory, antiseptic, anxiolytic and anti-oxidant properties (1, 6, 8, 14). Further studies emphasize its anti-viral, anti-cancer, and anti-immune functions (14,19). The pharmaceutical relevance of clove is consolidated with the usage of the product in food preservation, oral hygiene, aromatherapy, perfumery and traditional medicine(32). Both *P. niruri* and *S. aromaticum* have high potential to be developed into plant-based antimicrobial therapies due to their ethnomedicinal and commercial usefulness and because they have a great phytochemical proficiency. Their assessment is particularly applicable to LMIC context, to which treatment is not possible due to the problem of antimicrobial resistance and scarce drugs. The choice of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* as representative test organisms in the present study was done to ensure clinical relevance. Collectively, they provide a wide range of clinically significant pathogens, including *S. aureus* which is Gram-positive infections, *E. coli* which is Gram-negative enteric and uropathogen, and *S. typhi* which is a significant enteric pathogen in LMICs. Their use is useful as they offer an empirical model that can be used to evaluate both Gram-positive

and Gram-negative susceptibility and suit the study to the local infectious-disease burdens. This study, thus, examines the phytochemical composition and antimicrobial action of *Phyllanthus niruri* and *Syzygium aromaticum* in extracts of ethanol, boiled-water and distilled-water. The results will serve to confirm their historical application, to describe their biological activity and determine their potential as complements/alternatives antimicrobial agents.

Materials and Methods

Phyllanthus niruri (Sample A) leaves and fruits were obtained around the edge of the Tono irrigation dam in the Upper East Region of Ghana. The plant grows well in a waterlogged area. The choice of this site was due to the large number of plants in the area and the favorable environment to have the plant. In the case of *Syzygium aromaticum* (Sample B), the specimen was bought at the Navrongo marketplace, where it is fresh and mature, and as a representative of plant material, it is normally utilized in traditional medicines. The samples in all containers were sealed off in clean and black polyethylene bags to prevent contamination and were transported back to the laboratory in ambient conditions. The processing was done without delay to maintain the integrity of the phytochemicals to avoid degradation of the phytochemicals by microbes.

Sterilization and Disinfection

Lab glassware was thoroughly washed and sterilized by using a hot air oven at 160°C for 2 hours. The plastic materials and liquid reagents were autoclaved at 121°C, 15 minutes under 15 Psi. The 100% ethanol was used to disinfect laboratory benches, equipment such as electric blenders prior to and after use. Other tools like inoculating loops and metal borers were flame sterilized via a Bunsen burner just in time. Washing under running tap water incurred the removal of soil and debris in plant materials and was followed by the use of distilled water to reduce the level of microbial contamination.

Preparation of media

There was preparation of Mueller Hinton Agar (MHA) and Nutrient Broth based on the general microbiological procedures in order to have consistency and reliability in the course of bacterial growth and sensitivity testing. In making MHA, 38 g of the medium in the form of powder was dissolved in 1 L of distilled water, and heated by stirring thoroughly until the agar dissolved completely. This was followed by autoclaving of the mixture at 121°C of 15 minutes. The agar was then autoclaved; the molten agar was then left to cool to about 45°C after which it was poured into sterile Petri dishes to obtain a consistent agar depth of 4 mm. The prepared medium was adjusted to the final pH of 7.3 +- 0.1 to be able to comply with CLSI standards. Nutrient broth was made by dissolving 10 g of peptone, 10 g of beef extract and 5 g of sodium chloride (NaCl) into 500 mL of distilled water and then gently heating it which made the nutrient broth dissolve completely. Distilled water was thereafter added to the volume to 1 L. Broth was autoclaved at 121degC during 15 minutes and left to cool down. Thanks to the adjustment to the final pH of the broth at 7.2 +- 0.2, it was possible to obtain optimal bacterial growth when preparing the culture.

Test Organisms Preparation

In the present study selected *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi* which served as representative test organisms that are in pure laboratory culture. The bacteria were all aseptically inoculated in sterile nutrient broth and left to proliferate at 37°C for a movable 18-24 hours, which terminates as a harvest of fresh, easily expanding bacteria. This was to guarantee that the cultures reached the logarithmic (log) growth phase in which bacterial cells exhibit optimum cellular metabolism and maximum profusion to antimicrobial agents. The resultant turbidity of the broth culture was then matched to the 0.5 McFarland turbidity standard and where 0.5 McFarland turbidity standard was the approximated 1.5 x 10^8 CFU/mL. The comparison of the turbidity of the culture visually to the McFarland reference tube, with adequate light and correction by the sterile saline/broth where necessary, was used to standardize it. Any additional testing of antimicrobial sensitivity on the same was performed on such homogenate bacterial suspensions.

Leaves and Fruits Extract Preparation

Air-dried leaves and fruit of *P. niruri*, flower buds of *S. aromaticum* were weighed and crushed to fine powder. Three extracts of ethanol, distilled and boiled water were made. A single gram of powder was added to 10 mL of solvent to each extract and allowed to stand after five days at room temperature. Air drying was done to eliminate the ethanol. All extracts were kept in airtight containers in a sterile and labeled using the same type.

Preparation of the antibiotics

The process of testing antimicrobial susceptibility used 2 common antibiotics which are Cefuroxime and Ciprofloxacin against which the plant extracts would be compared. The necessary volume of each antibiotic was dissolved in sterile distilled water with a high level of precision and resulting in stock solutions that contained a homogeneous amount of the required antibiotic. These antibiotics have been chosen on the basis that they are popular broad-spectrum agents against Gram-positive and Gram-negative microorganisms, and hence they would form good control groups against which the antimicrobial efficacies of the plant extracts would be contrasted. Ready antibiotic solutions were the positive controls, and it is in this regard that the comparison of the inhibition zones of the plant extracts with the already known antimicrobials was made possible. This gave sufficient justification to the results obtained in the experiment and also gave a point of reference in the analysis of extract potency.

Antibacterial Sensitivity testing of the extracts

The determination of the antibacterial activity of the extracts was done by the agar well diffusion technique. A 0.1 mL of the standardized bacterial suspension was evenly inoculated onto new plates of Mueller-Hinton Agar (MHA) plates. Each plate was aseptically punctured using a sterile 6 mm metal borer 5 times. The different volumes of the plant extract were assigned to three wells, where the remaining two served as the control of the antibiotics. The antibiotic solutions and the plant extracts were spotted as per the measurement of the volumes and allocated to the individual wells. The plates were allowed to be at room temperature to ensure that the test substances could diffuse into the agar permanently. They were incubated for 24 hours at 37°C. The zone of inhibition of each well was measured in millimeters after incubation using a transparent graduated ruler and finally checked using a colony counter to increase accuracy and reproducibility.

Phytochemical Analysis

The standard procedures carried out to determine the presence of alkaloids, flavonoids, saponins, phenols, and steroids in extracts were considered as qualitative phytochemical screening of extracts. Later alkaloids were observed using the Wagner reagent (reddish-brown precipitate). The test using an alkaline reagent confirmed the presence of flavonoids (the yellow color changed to colorless by adding NaOH). On vigorous shaking, saponins were observed in the form of persistent frothing. Phenols produced a dark green color with ferric iron chloride. The reaction with chloroform-sulfuric acid was used to determine the presence of steroids, which gave a positive result, resulting in stable color changes. The tests were conducted thrice, so as to achieve retest consistency.

Statistical Analysis

The data was run on Microsoft Excel and IBM SPSS (v26). To compare the mean zones of the inhibition of the plant extracts and antibiotic controls, one-way ANOVA was employed, whereas the Tukey post-hoc test was applied to conduct the comparison of the data. The P-values below 0.05 were considered significant. A graphical representation of the results in terms of bar charts was made in order to visualize the comparative efficacy and variability, whereby the error bars were employed to reflect the standard deviation.

Results and Discussion

Table 1 demonstrates plant samples and extracts that was used in antimicrobial and phytochemical research. Sample A (leaves and fruits), *Phyllanthus niruri* and Sample B (flower bud), *Syzygium aromaticum* were subjected to different conditions. Three methods were used to extract each namely; ethanol, boiled water, and distilled water. In the case of Phyllanthus niruri, the air-dried (A1-A3) and fresh (B1-B3) samples were used, but *Syzygium aromaticum* was processed in a similar manner (C1-C3). This coding system (A1-C3) offers a direct source of reference and association, which links each extract towards its mode of preparation and plant source, thus resulting in uniformity in the interpretation of the experimental findings

Antibacterial activity of Ethanolic extracts on Test Organisms

Table 2 reveals that the mean of the zones of inhibition (mm) of the three test organisms *(Salmonella typhi, Escherichia coli and Staphylococcus aureus)* were 0.3110, 0.3065 and 0.3010 respectively with the use of ethanolic extracts namely A1, B1 and C1. In *Salmonella typhi*, happens to be the highest inhibition (12.75 +- 1.5 mm) recorded by extract A1 with C1 coming close with

(12.5 +- 1.91 mm), and B1 having a slightly smaller amount (11.25 +- 3.0 mm). On Escherichia coli A1 had a 12.0 +- 0 mm inhibition zone, B1 produced 11 +- 0 mm and C1 produced 10 +- 1.41 mm. In *Staphylococcus aureus* extract C1 gave the highest zone of inhibition; of 15.25 +- 3.0 mm, B1; of 12.75 +- 2.22 mm and A1; of 11.25 +- 2.36 mm. The table contains an overall comparative study of the ethanol extracts on the performance on the chosen organisms in terms of antibacterial activity.

Antibacterial activity of Boiled water extracts on Test organisms

Table 3 shows the average zone inhibition (mm) of the boiled water extracts A2, B2 and C2 against *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus*. In the case of *Salmonella typhi*, extract A2 gave the most inhibition zone (13.25 +- 0.96 mm), whereas C2 exhibited intermediate activity (11.5 +- 0 mm). There was no inhibition (0.00 mm) in extract B2. Against *Escherichia coli*, only extract C2 (10.75 +- 0.35 mm) inhibited, whereas A 2 and B 2 were not active (0.00 mm). In the case of *Staphylococcus aureus*, extract B2 had the highest inhibition (19.625 +- 6.75 mm), then C2 (11.75 +- 0.35 mm). No inhibition was found with A2 of extract (0.00 mm). The table gives an overall report on the antibacterial behavior of the boiled water extracts with indication of the variations in the activities of the test organisms.

Antibacterial activity of Distilled water extracts on Test organisms

Table 4 shows the mean of the zone of inhibition (mm) of the distilled water extracts A3, B 3 and C3 against the test organisms *Salmonella typhi, Escherichia Coli* and the *Staphylococcus aureus*. In the case of Salmonella typhi, extract B3 had the highest measure of activity (13.625 +- 2.06 mm), next was extract A3 (9.5 +- 1.41 mm), and the least activity was recorded in extract C3 (7.875 +- 2.95 mm). Against the *Escherichia coli*, extract C3 had the greatest activity (14.75 +- 1.5 mm), followed by A3 (13.5 +- 2.12 mm

Antibacterial Test organisms Control (Ciprofloxacin and Cefuroxime) Results

Table 5 shows the mean areas of inhibition (mm) effected by the control antibiotics of cefuroxime and Ciprofloxacin (both of 1 mg/mL) on the test organisms *Salmonella typhi, Escherichia coli*, and *Staphylococcus aureus*. Cefuroxime gave an inhibition zone of 12.5 +- 2.2 mm against *Salmonella typhi* and Ciprofloxacin recorded a significant inhibitory zone of 27.0 +- 0 mm. Cefuroxime had an inhibition zone of 16.0 +- 5.1 mm against *Escherichia coli* and Ciprofloxacin had an inhibition zone of 35.0 +- 0 mm. In the case of *Staphylococcus aureus*, Cefuroxime gave an inhibition zone of 12.5 +- 2.2 mm, which was once again highest in Ciprofloxacin of 35.0 +- 0 mm. The table summarizes the performance of the standard reference antibiotics being used as the controls in the study in terms of the antibacterial activity.

Findings of the primary phytochemical screening of *Phyllanthus niruri* and *Syzygium*

The table indicates the presence or absence of the desired phytochemical constituents (+/-), and the indicators of the reactionity of the constituents. Both samples of plants had tested positive in alkaloids as a reddish-brown precipitate was formed. Both samples had saponins, which were supported by the persistence of the foam. Flavonoids were identified in both samples that yielded a colorless reaction. The presence of phenols was observed in the two plant extracts indicated by the visualization of dark green color. The two samples also carried steroids, which gave them a yellow and green fluorescence on the upper layer that was red. The results of the phytochemical profile of the two samples are presented in the table that the phytochemicals tested were all found in both the plants.

Efficacy of Extract against the Selected Bacteria

The bar graph and the title Efficacy of Extracts Against Microbes represents the comparison in antimicrobial activity of nine extracts (A1-C3) on Salmonella typhi, Escherichia coli and Staphylococcus aureus. Each extract is represented as a row of three bars with a color code: blue, indicating S. typhi, orange, indicating E. coli and grey, indicating *S. aureus* and the heights of the bars indicate mean zones of inhibition, which is the evidence of antimicrobial potency. Throughout the graph, there are distinctions in their activity: some extracts demonstrate broad-spectrum, jeopardizing all three bacteria with fairly wide territories whereas others exhibit selective activity, which reaches a certain pathogen and specifies it. The error bars demonstrate the variations of an

experiment, and they provide the reliability of the data as well as its interpretation. On the whole, the graph shows the presence of extracts with high antimicrobial properties, indicates variations in the susceptibility of bacteria, and indicates that it is possible that more studies should be conducted on the chemical content of each extract. The results offer a foundation on the exploration of specific natural therapy or disinfectants, with a focused interest on the usefulness and the therapeutic potential of the sampled plant extracts.

Figure.1 Representation of Mean Zones of Inhibition

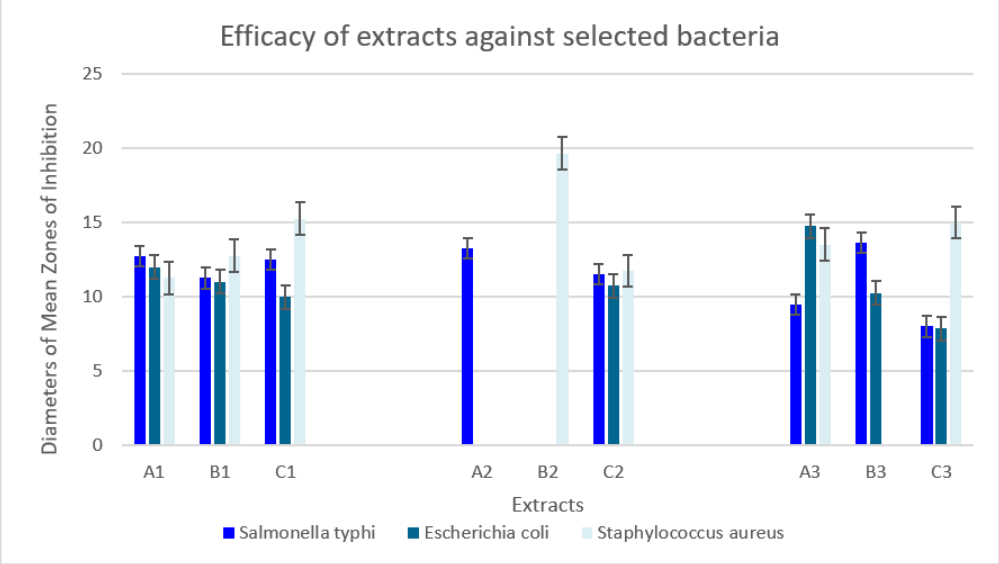

Table.1 Definition for the Samples and Various Extracts

Definition	Samples
Sample A	Leaves and fruits of *Phyllanthus niruri*
Sample B	Flower but of *Syzygium aromaticum*
A1	Ethanolic extract for air-dried *Phyllanthus niruri*
B1	Boiled water extract for air-dried *Phyllanthus niruri*
C1	Distilled water extract for air-dried *Phyllanthus niruri*
A2	Ethanolic extract for fresh *Phyllanthus niruri*
B2	Boiled water extract for fresh *Phyllanthus niruri*
C2	Distilled water extract for fresh *Phyllanthus niruri*
A3	Ethanolic extract for air-dried *Syzygium aromaticum*
B3	Boiled water extract for air-dried *Syzygium aromaticum*
C3	Distilled water extract for air-dried *Syzygium aromaticum*

Table.2 Antibacterial activity of Ethanolic extracts on Test Organisms

Mean diameters of Zones of Inhibition (mm) for Ethanolic extracts			
Test Organisms	A1	B1	C1
Salmonella typhi	12.75 ± 1.5	11.25 ± 3.0	12.5 ± 1.91
Escherichia coli	12.0 ± 0	11 ± 0	10 ± 1.41
Staphylococcus aureus	11.25 ± 2.36	12.75 ± 2.22	15.25 ± 3.0

Table.3 Antibacterial activity of Boiled water extracts on Test organisms

Mean diameters of Zones of Inhibition (mm) for Boiled water extracts			
Test Organisms	A2	B2	C2
Salmonella typhi	13.25 ± 0.96	0.00	11.5 ± 0
Escherichia coli	0.00	0.00	10.75 ± 0.35
Staphylococcus aureus	0.00	19.625 ± 6.75	11.75 ± 0.35

Table.4 Antibacterial activity of Distilled water extracts on Test organisms

Mean diameters of Zones of Inhibition (mm) for Distilled water extracts			
Test Organisms	A3	B3	C3
Salmonella typhi	9.5 ± 1.41	13.625 ± 2.06	8 ± 1.41
Escherichia coli	14.75 ± 1.5	10.25 ± 1.26	7.875 ± 2.95
Staphylococcus aureus	13.5 ± 2.12	0.00	15 ± 0

Table.5 Antibacterial activity of Control (Cefuroxime and Ciprofloxacin) on Test organisms

Mean diameters of Zones of Inhibition (mm) for Controls (Cefuroxime and Ciprofloxacin)		
Test Organism	Cefuroxime (Control, 1 mg/mL)	Ciprofloxacin (Control,1 mg/mL)
Salmonella typhi	12.5 ± 2.2	27.0 ± 0
Escherichia coli	16.0 ± 5.1	35.0 ± 0
Staphylococcus aureus	12.5 ± 2.2	35.0 ± 0

Table.6 Preliminary phytochemical screening of Sample A and Sample B

Phytochemicals	***Phyllanthus niruri***	***Syzygium aromaticum***	**Inference**
Alkaloids	+	+	Reddish-brown ppt
Saponins	+	+	formation of persistent foam
Flavonoids	+	+	Colourless
Phenols	+	+	Dark green colour
Steroids	+	+	Red upper layer, Yellow with green fluorescence

+ = present - = absent

Table.7 ANOVA for ethanolic extracts

Extract Type	Source	F-value	p-value
Ethanolic	A1	0.862	0.455
	B1	3.245	0.087
	C1	7.250	0.013
Boiled Water	A2	766.091	<0.000
	B2	33.812	<0.000
	C2	0.006	0.994
Distilled Water	A3	3.528	0.074
	B3	103.946	<0.000
	C3	0.518	0.612

Table.8 ANOVA for ethanolic extracts

		Sum of Squares	Df	Mean Square	F-value	P-value
A1	Between Groups	4.50	2	2.250	.86	0.455
	Within Groups	23.50	9	2.611		
	Total	28.00	11			
B1	Between Groups	117.17	2	58.583	3.25	0.087
	Within Groups	162.50	9	18.056		
	Total	279.67	11			
C1	Between Groups	225.17	2	112.583	7.25	0.013
	Within Groups	139.75	9	15.528		
	Total	364.92	11			

Table.9 ANOVA for ethanolic extracts

		Sum of Squares	Df	Mean Square	F-value	P-value
A2	Between Groups	468.17	2	234.083	766.09	0.000
	Within Groups	2.750	9	.306		
	Total	470.92	11			
B2	Between Groups	1027.04	2	513.521	33.81	.000
	Within Groups	136.69	9	15.188		
	Total	1163.73	11			
C2	Between Groups	.542	2	.271	0.006	.994
	Within Groups	386.13	9	42.903		
	Total	386.66	11			

Table.10 ANOVA for boiled water extracts.

		Sum of Squares	df	Mean Square	F-value	P-value
A3	Between Groups	224.000	2	112.00	3.528	0.074
	Within Groups	285.750	9	31.750		
	Total	509.750	11			
B3	Between Groups	402.792	2	201.39	103.946	0.000
	Within Groups	17.438	9	1.938		
	Total	420.229	11			
C3	Between Groups	36.542	2	18.27	0.518	0.612
	Within Groups	317.188	9	35.24		
	Total	353.729	11			

The mean difference is significant at the 0.05 level

ANOVA of antibacterial activity of the different extracts

Table 7 shows findings of ANOVA on the antibacterial activity of ethanolic, boiled water and distilled water extract on the test organisms. The table indicates the extract types (ethanolic, boiled water, distilled water), and individual extracts (A1-C3), extracts F-values and p-values. In the case of ethanol extracts, A1 and B1 had no significant difference in terms of activity ($p = 0.455$ and 0.087 respectively; however, C1 had an acceptable significance value of $F = 7.250$, $p=0.013$). Extract of A2 and B2 boiled water had a very important activity ($p < 0.000$), C2 did not have any significant effect ($p = 0.994$). B3 exhibited very high significant antibacterial activity among the distilled water extracts ($F = 103.946$, $p < 0.000$), whereas A3 and C3 did not have any significance ($p = 0.074$ and 0.612, respectively). A comparative summary of statistical significance of both extracts is presented in the table, on which the extracts that demonstrated significant differences in the values of antibacterial efficacy.

There were noticeable differences observed in the antibacterial activity of the extracts of *Phyllanthus niruri* and *Syzygium aromaticum* which differed with the part of plant, the method of extracting, and the bacteria being tested. The inhibitory effects of ethanolic extracts tended to be higher as compared to aqueous extracts. This was in line with previous research that indicated that the number of phenolic and non-polar antimicrobial constituents extracted by ethanol was greater than those extracted by water and thus this led to increased antibacterial activity (2, 12, 16). The ethanolic extract of *S. aromaticum* (C1) was the strongest among the extracted extracts especially when it came to the *Staphylococcus aureus* (15.25 +- 3.0 mm). This is congruent with the research of Adefegha and Oboh (1), Chaieb *et al*. (6), and Hasan *et al* (14), who indicated that the high trait of antimicrobial activity of clove is due to its high content of phenolic and flavonoid compounds, which include eugenol. Your findings are supported yet again by similar research by Gupta *et al*. (13) and Divya *et al*. (9), who also mention the abundance of phytochemistry in *P. niruri* as the source of its antibacterial activity. Boiled water extracts, including *P. niruri* (B2), were selective inhibitors, and strong against *S. aureus* but weak against *E. coli* and *S. typhi*. This trend is similar to the results of Kaur *et al*. (18), Kumar *et al*. (21), who stated that aqueous extractions were not always effective in the extraction of non-polar antimicrobial compounds, leading to weak activity against Gram-negative bacteria because of the complexity of their outer membrane. On the same note the moderate antibacterial properties exhibited by the distilled water extracts reflects what was observed in the past by other reports that indicated that the capacity of the solvent to extract is strongly tied to its polarity and the methodology used during extraction (2, 16). ANOVA findings also corroborated these results and showed a significant difference between specific extracts (C1, A2, B3) also indicating the importance of extraction

conditions on the level of antimicrobial activity. Similar decisions were reached in works, which investigated solvent-dependent difference in phytochemical activity (12, 16). The presence of the alkaloids, flavonoids, saponins, phenols, and steroids were confirmed through screening of phytochemicals. These types of metabolites are well known regarding their antimicrobial action, which contains membrane disruption, protein synthesis, DNA interference as well as deprivation of vital metal ions (1, 6, 9, 13,). Similarly, studies by Salehi *et al*. (29) and Mishra *et al*. (23) also lay the blame of the antibacterial activity of plant extracts on the summative or synergistic activity of several phytochemicals and recommend the variability of your extracts. Regardless of the standard antibiotics, especially ciprofloxacin, which showed significantly larger zones of inhibition, your results and literature support the view that, notwithstanding being weaker, the use of plant extracts as a complementary antimicrobial agent, as well as a source of new drug development, is of value (5, 7, 24,). In particular, Chanda and Rakholiya (5) emphasized the need to use natural products in conjunction with conventional antibiotics to fight resistance, a strategy supported by world reviews on antimicrobial resistance by Prestinaci *et al*. (27) and O'Neill (26). In general, this research validates that *P. niruri* and *S. aromaticum* have excellent medicinal properties, in accordance with the findings of Kamboj (15), Qazi and Molv (28), and WHO (33), on the usefulness of medicinal plants in medical practices. The proven antibacterial properties support the need to conduct additional studies on the isolation of active compounds, their structure, and the examination of their activity in order to assist in the creation of new antimicrobial treatment.

In conclusion, this paper attempted to test the phytochemical constituents and the antimicrobial activity of the extract of the leaf and fruit of *Phytanthus niruri* and the flower bud of *Syzygium aromaticum* being extracted using ethanol, distilled water and boiled water as the extraction solvents. The findings demonstrated the presence of major phytochemicals that is, the phenols, alkaloids, saponins, steroids, and flavonoid compounds in the various extracts. The accumulation of both the *P. niruri* and *S. aromaticum* was observed to have considerable broad-spectrum antibacterial activity with regards to the selected bacterial species, and the antimicrobial effect of the two plants was probably due to the identified bioactive compounds. Therefore, the results validate the use of such plants in traditional medicine to treat bacterial infections. Furthermore, an extract mixture of the two herbs proves that it is possible to develop effective herbal medicines, which can manage the largest variety of bacterial diseases.

Acknowledgments

The authors give their deepest appreciations to the management and laboratory staff of C.K Tedam University for Technology and Applied Sciences, Department of biological sciences for assisting and cooperating with them during the course of the study. Special appreciation is also given to all participants whose goodwill to respond to the queries has caused this research.

Author Contributions

Ebenezer Yeboah- Conceptualization, Resources, Investigation, writing original draft, Methodology Samuel Kwarteng- Data curation, Formal analysis, Validation; Lawrence Adetunde – Project administration, Supervision, Writing-review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Ebenezer Yeboah, Samuel Kwarteng and Lawrence Adetunde. 2026. Comparison of antimicrobial effect and phytochemical profile of *Phyllanthus niruri* and *Syzygium aromaticum* extracts against some selected bacteria. *Int.J.Curr.Microbiol.App.Sci.* 15(1): 194-205. **doi:** https://doi.org/10.20546/ijcmas.2026.1501.024