

Original Research Article

Phytochemicals and Antibiotic Studies of Ethanolic Extracts of *Dennettia Tripetala* (Mmimi/Pepper Fruit) and *Psidium Guajava* (Guava) Leaves on Clinical Isolates of *Staphylococcus Aureus* and *Escherichia Coli*

Ozor Joseph Okwudilichukwu^{1*}, Anyamene Christopher Okwudili²

¹Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Nigeria

²Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Nigeria

*Corresponding Author

Ozor Joseph Okwudilichukwu
Department of Applied Microbiology
and Brewing, Enugu State University
of Science and Technology, Nigeria

Article History

Received: 08.06.2024

Accepted: 15.07.2024

Published: 22.07.2024

Abstract: Aqueous and ethanolic leaf extracts of *Dennettia tripetala* and *Psidium guajava* were evaluated for their antimicrobial effects against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* from Annunciation Specialist Hospital Enugu State Nigeria. The identified isolates were sub-cultured on sterile nutrient agar plates and incubated over night at 37°C. The identified isolates were then re-identified and characterized. Polymerase chain reaction (PCR) was used to detect *Staphylococcus aureus* strain which has 85% pairwise similarity with *Staphylococcus haemolyticus* strain SH1 and *Escherichia coli* strain which has 99.62% pairwise similarity with *E. coli* strain AC1. Quantitative and qualitative phytochemical analysis were done on the extracts of *Dennettia tripetala* and *Psidium guajava* using water and ethanol as extracting solvents. The phytochemical screening of the extracts revealed the presence of tannins, saponins, alkaloids, flavonoids, glycosides and phenols. Quinonones however, was absent in this study. The isolates were screened for sensitivity to different extracts using agar well diffusion technique. The results indicated that both the ethanol and aqueous extracts of the *Psidium guajava* leaves showed inhibitory activity against *Staphylococcus aureus* at 10mm and 12mm respectively but no activity against *E. coli*. On the other hand, the aqueous extracts of the *Dennettia tripetala* showed inhibitory activity against *S. aureus* and *E. coli* at 20mm and 10mm respectively whereas, the ethanol extract has no activity on both test microorganism. The extracts were reconstituted with Dimethyl sulfoxide (DMSO) to concentrations (mg/ml) of 200, 100, 50, 25, 12.5 and the minimal inhibitory concentration (MIC) of the extracts were determined using agar well method. The MIC of the *Psidium guajava* leaves extract revealed that both ethanolic and aqueous extracts inhibited *S. aureus* at 12.5mg/ml. While MIC of the *Dennettia tripetala* revealed that the aqueous extract inhibited *S. aureus* at 50mg/ml and *E. coli* at 100mg/ml from the results, the aqueous extracts of both plants generally have a better inhibitory property than the ethanolic extracts. Based on the present findings, *Dennettia tripetala* and *Psidium guajava* leaves possess the capability of being good candidates in the search for a natural antimicrobial agent against infections or diseases caused by *Staphylococcus aureus* and *Escherichia coli*.

Keywords: Phytochemicals, Antibiotics, *Dennettia Tripetala*, *Psidium Guajava*, *Staphylococcus aureus*, *Escherichia coli*.

Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

Citation: Ozor Joseph Okwudilichukwu & Anyamene Christopher Okwudili (2024). Phytochemicals and Antibiotic Studies of Ethanolic Extracts of *Dennettia Tripetala* (Mmimi/Pepper Fruit) and *Psidium Guajava* (Guava) Leaves on Clinical Isolates of *Staphylococcus Aureus* and *Escherichia Coli*. *Glob Acad J Med Sci*; Vol-6, Iss-4 pp- 181-189.

INTRODUCTION

About 80% of the world population relies chiefly on traditional medicines for their primary health care needs [1]. Studies have shown that bioactive natural compounds exhibiting antimicrobial activities have been isolated mainly from cultivatable microbial strains [2].

The different parts of the plant have been used in traditional medicinal practice in the treatment of various infections in Nigeria. Essential oil from its leaves has been reported to possess significant antibacterial and anti-inflammatory activities [3].

Extracts of the root and leaves are commonly used in folk medicine in combination with other medicinal plants to treat various ailments including fever, infantile convulsion, typhoid, worm infestation, vomiting and stomach upset. It has also been reported that the essential oil and phenolic acid extract of *Dennettia tripetala* can inhibit the growth of food-borne micro-organisms [4]. Studies have revealed that the leaves were effective in inhibiting the growth of the rot-causing fungus *Sclerotium rolfsii* in cocoyam both in vitro and in vivo [5].

Psidium guajava L. (guava) (Myrtaceae), is a native plant of tropical America, but it is now cultivated all over the world especially in the tropical regions like Nigeria. Pharmacological investigations indicated that its bark, fruit and leaves possess antibacterial, hypoglycemic, anti-inflammatory, antipyretic, asmolitic and central nervous system depressant activities [6]. It contains a number of major bioactive constituents like flavonoids, guayavolic acid, guavanoic acid, guajadial, guajaverin and so many others that may account for these medicinal and pharmacological activities. The methanol root extract was found to be fungicidal [7].

The *Psidium guajava* leaf extracts have trypanocidal activity which may be attributed mainly due to the broad antimicrobial property of the flavonoids and the iron chelating property of tannins [8]. The in vitro antibacterial activity of *Psidium guajava* L. leaf extract on *Staphylococcus aureus* was possibly due to protein degrading activity of the extracts [9]. Four antibacterial compounds were isolated from the leaves of *Psidium guajava* and the flavonoids extracted from guava leaves were found to be active against the several strains of spoilage and food borne pathogenic bacteria [10].

Due to the minimal research on this study, this research will therefore, investigate and compare the antimicrobial and antibiotic activities of ethanol and aqueous leaf extracts of *Dennettia tripetala*

(pepper fruit) and *Psidium guajava* (guava) in order to justify their use in traditional medicinal practice. This is with a view of enrolling them to alleviate the current health care challenges, such as increasing cost of chemotherapy, emergence of multidrug resistant microbial strains and unmet clinical outcome [11].

EXPERIMENTAL SECTION

MATERIALS AND METHODS

Sterilization of Glass wares

All glass wares were soaked in water with detergent for 30 minutes then washed and scrubbed with brush. After that, it was rinsed with clean tap water. The glass wares were allowed to dry and the mouth of test tubes and conical flasks were stock with cotton wool then all the glassware were wrapped with aluminum foil. After this, the wrapped glassware were sterilized in hot air oven at 160°C for one hour then allowed to cool before use.

Collection of Leaf Samples

Fresh leaves of *Dennettia tripetala* (pepper fruit) and *Psidium guajava* (guava) were harvested from Okpatu town in Udi local government area of Enugu State, Nigeria and authenticated by Mr Alfred Ozioko a taxonomist with Bioresource Development and Conservation Program (BDCP) Research Center Nsukka, Enugu State, Nigeria. The plants were sorted and washed with clean water and allow to shade dry for 3 weeks. Then the dried plants materials were ground into powder using sterile electric grinder to coarse powder.

Preparation of Media

Nutrient Agar: 7g of nutrient agar powder was weighed out with weighing balance and dissolved in 250ml of distilled water in a conical flask and agitated vigorously, and then it was autoclaved at 121°C for 15minutes. After that, it was allowed to cool to 40°C then it was poured into Petri dish for use. This was done according to manufacturer's guide.

Mueller Hinton Agar: Following the manufacturer's guide, 11.4g of Mueller Hinton Agar powder was weighed out with weighing balance and it was dissolved in 300ml of distilled water contained in conical flask and agitated vigorously. The mouth of the conical flask was corked with aluminum foil then autoclaved at 121°C for 15minutes. It was allowed to cool to 40°C before it was poured into the plate and set for further use.

Peptone water: Following the manufacture's guide, 3.75g of peptone water was weighed out with weighing balance and it was dissolved in 250ml of distilled water in a conical flask and agitated vigorously. The mouth was corked with aluminum

foil. It was autoclaved at 121°C for 15 minutes, after that it was allowed to cool to 40°C before it was poured into a sterile tube.

(EMB) Eosin Methylene Blue Agar: 9g of EMB agar powder was weighed out with weighing balance and dissolved into 250ml of distilled water following the manufacturer's guide; then agitated vigorously and the conical flask was corked with aluminum foil. After that, it was autoclaved at 121°C for 15 minutes. It was allowed to cool at 40°C before it was poured into plate for further use. Prepared according to the manufacturer's guide.

Preparation of McFarland Solution with Standardization of Inoculums

0.5 McFarland solution was prepared by mixing 0.05g of 1.75% barium chloride dehydrate (BaCl₂.2H₂O) with 9.95ml of 1% sulphuric acid (tetraoxosulphate VI acid, H₂SO₄) in a test tube. 10ml of normal saline were placed on freshly sterilized test tube and it was incubated with the testing bacterial each on a single tube containing 10ml of saline and mash it with McFarland solution. This followed the manufacturer's guide.

Extraction of the Plant Materials

Ethanol (99%) and cold water extract as described in AOAC [12] was adopted for this study. 30gram of each of plants sample were weighed out and dissolved into sterile 300ml conical flask containing 200ml of ethanol and cool water in respective conical flasks. And it was agitated well for 30 minutes then allowed standing for 48 hours. After that it was filtered using Whatman's filter paper and evaporated using water bath at 60°C for 3 hours. Then the concentrate samples were stored in refrigerator for phytochemical and antibacterial analysis.

Preparation of Test Organisms

The chemical isolates of *Staphylococcus aureus* and *Escherichia coli* were collected from the microbiology department of Annunciation Specialist Hospital, Emene Enugu from (UTI) Urinary tract Infection. The identified isolates were then sub-cultured on sterile nutrient agar plates and incubated overnight at 37°C. The identified isolates were then re-identified and characterized. The microbial cultures were diluted with peptone water until the final suspension contained about 1.5x10⁸cfu/ml of *Staphylococcus aureus* and *Escherichia coli* according to the method of Akujobi *et al.*, [13]. The cell densities obtained were in accordance with 0.5 McFarland's standard and used in all the investigations. The McFarland was prepared by adding 0.1ml of BaCl₂ unit 9.9ml of 1% Sulphuric acid.

Phytochemical Analysis

Alkaloid Test: 2ml of each of the extracts were added into test tube and 5ml of 1% aqueous hydrochloric acid was added then placed in a water bath for 3 minutes. Thereafter, 3 drops of Mayer's reagent was added. A white precipitate formed, indicates presence of alkaloid while none indicates absence of alkaloid [14].

Flavonoid Test: 2ml of each extracts were added into different test tubes and 4 drops 10% of NaOH solution was added into tubes containing the extracts. A yellow colour observed which shows the presence of flavonoid while no yellow colour shows absence of flavonoid.

Tannins Test: The 1ml of each of the extracts was added into different test tubes and 2ml of 1% ferric chloride solution was added into the tubes. A greenish colour change shows the presence of tannins while no colour change shows absence of it.

Saponins Test: 10ml of distilled water was added into a test tube containing 2ml of different leaves extracts each and agitated vigorously [12]. Persistent frothily even after heating is an indication of the presence of saponins but no frothily even after heating is an indication of absence of saponins.

Phenol Test: 5ml of each of the extracts were added into the test tubes containing 8ml of distilled water and 6ml of ferric chloride was added to each of the mixture. A colour change to light brown indicates a positive test while none indicates a negative result [15].

Cardiac Glycoside Test: 1ml of the extracts was placed into a test tube each. 2ml of chloroform was added and then 2ml of concentrated tetraoxosulphate (vi) acid was added to form a lower layer. A reddish brown cob at the inter phase is an indication of a positive test while none is an indication of a negative test.

Antrojanone Test: 2ml of each of the extracts and 5ml of 10% ammonia was added into then test tubes and agitated vigorously. 2ml of benzene was there after added. A colour change is an indication of a positive test while none is an indication of a negative test [14].

Re-Identification and Characterization of Bacteria Gram Staining Method

Make a thin smear, allow to air dry, Heat fix by passing slide 3-4 times through the flame of a Bunsen burner, Cover smear with a primary stain crystal violet for 45-60s, Rinse with water, Cover smear with mordant Lugol's iodine for 45-60s, rinse

again, decolourise with acetone for 1-3s, rinse and counterstain with safranin for 1-2mins, rinse, place in a slanting position to air dry. Examine with 40x objective lens [16].

Motility Test

With a sterile straight needle, a colony of a young (18 to 24 hour) culture growing on agar medium was touched. A single stab was made down the center of the tube to about half the depth of the medium. This was incubated at 35°-37°C and examined daily for up to 7 days. The result is positive when a diffuse, hazy growths that spread throughout the medium rendering it opaque is observed. It is negative when the growth is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent [17].

Biochemical Test

This was done according to Nwadioha *et al.*, [16]

Catalase

Emulsify the test organism with 1 or 2 drops of hydrogen peroxide on a glass slide. An observed bubbling of air effect indicated oxygen gas production and such bacteria is said to be catalase positive.

Coagulase

Make a dense suspension of the organism using 1-2 drops of distilled water on a glass slide (Observe for auto agglutination). Mix with a loopful of EDTA plasma and observe for clumping or agglutination.

Oxidase

Add 2-3 drops of oxidase reagent to a filter paper and smear a colony of test organism on it using a piece of glass rod. A positive result shows the dark purple colour within 30seconds.

Indole

Inoculate a bijou bottle containing peptone water with the test organism. Incubate at 35-37°C for 24-48 hours. Then add 0.5mls of kovac's reagent. Shake gently and examine for a red colour on the surface. The formation of pink to red ring colour indicates positive test while no colour change indicates negative test.

Molecular Test

Polymerase chain reaction was done using *Staphylococcus aureus* (S1) and *Escherichia coli* (S2). A sample each was sent for DNA characterization and sequencing at Bioformatic Services Ibadan Nigeria.

Antimicrobial Sensitivity Testing of the Plant Extracts

The agar well diffusion technique as described by Balouiri *et al.*, [18] was adopted for the study to evaluate the antibacterial activity of the extract. The standardized inocular culture of the respective test bacteria was spread evenly on the surface of the Mueller Hinton agar plates. Wells of 6mm were aseptically punched on the agar using a sterile agar cork borer allowing 30mm between adjacent wells on the petri dish. 0.2ml aliquot of each of the extracts was dropped into the agar holes and appropriately labeled. Antibiotic disc of Ciprofloxacin 30mg was used as control in each case. The Mueller Hinton agar plate was then incubated at 37°C for 24hours. The zones of inhibitions were measured with a meter rule.

Test for Minimum Inhibitory Concentration of the Plant Extract

Minimum Inhibitory Concentration (MIC)

Two fold serial dilutions were carried out as described by NCCLS [19]. 2ml of each leaves extracts were dissolved in 4ml of sterilized peptone water. These give 200mg/ml. Therefore, the two fold serial dilution was carried out from the stock solution 200mg/ml concentration by set of five test tubes on the test rack and 2ml of peptone water were added into each of the tubes respectively. 2ml from the stock was taken and transferred into the first tube containing 2ml of peptone water with fresh syringe with needle and it was homogenized properly. Fresh syringe with needle was used to transfer 2ml from the first tube of dilution and added into the second tube and homogenized properly. This procedure of transferring 2ml from the tube to 2ml of peptone water contained in the subsequent tube was continued till the fifth tube. The following concentration were there after obtained; 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. Having obtained the different concentration and dilution, three drops of from the standardized organism were inoculated into the various dilution of each case of the test organisms [13]. 2ml of mc-farland solution with 2ml of peptone water and 2ml of peptone water with three drops of the testing organisms serve as control sample. The tubes were incubated at 37°C for 24hours. After that, the tubes were examined by viewing the tubes with naked eyes. (Clean solution and turbidity were examined).

Minimal Bacteria Concentration (MBC)

From the MIC result, the tubes that showed no visible growth were sub cultured onto sterile nutrient agar plate and incubated at 37°C for 24hours and the plates were examined. The presence of bacteria growth in the agar plate then recorded.

RESULTS

Table 1: Phytochemical composition of aqueous and ethanolic extracts of Pepper fruit and Guava leaves

Phytochemical Constituents	Aqueous extract		Ethanolic extract	
	Pepper Fruit	Guava	Pepper Fruit	Guava
Alkaoids	+	+	+	+
Flavoids	+	+	+	+
Tannis	+	+	+	+
Saponin	+	+	-	+
Phenols	+	+	+	+
Glycoside	+	-	+	-
Quinonas	-	-	-	-

Key: (+) = present of the constituents; (-) = absence of the constituents

Table 2: Biochemical Tests to confirm Test bacteria

Test Bacteria	Gram staining	Shape	Catalase	Coagulase	Oxidase	Indole
<i>Staphylococcus aureus</i>	+	Round	+	+	-	-
<i>Escherichia Coli</i>	-	Rod	+	-	-	+

Note: the (+) sign indicates a positive test while the (-) sign indicates a negative test.

Table 2 shows Biochemical Tests to confirm the presence of the Test bacteria organisms used. Gram staining was used to differentiate the two

organisms into gram positive and gram negative bacteria using the shape as indicated above.

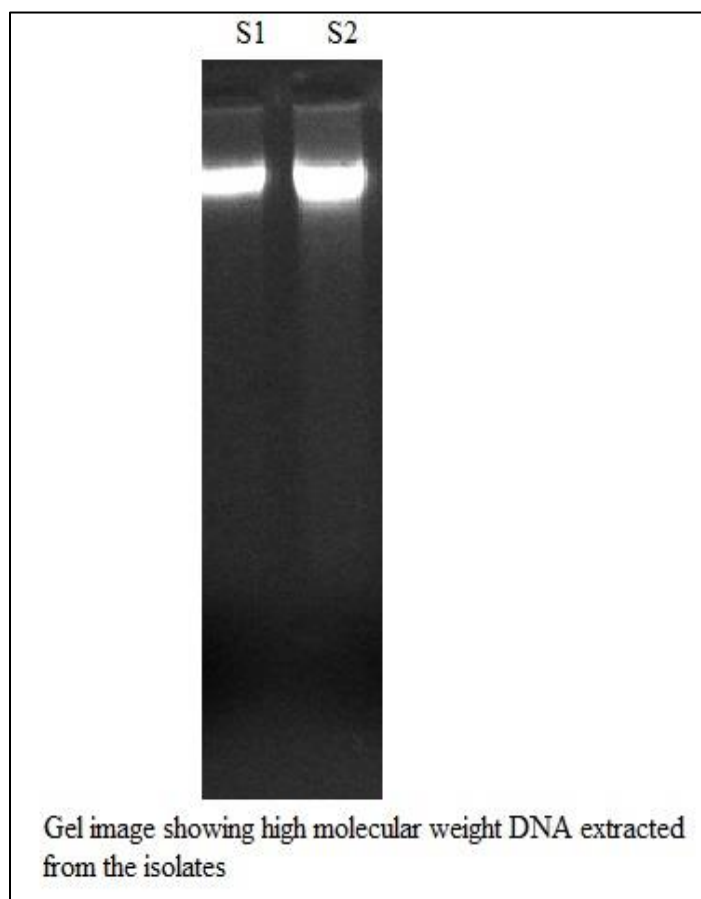


Figure 1

>S1 has 85% pairwise similarity with *Staphylococcus haemolyticus* strain SH1 which has NCBI accession number MK886483.1

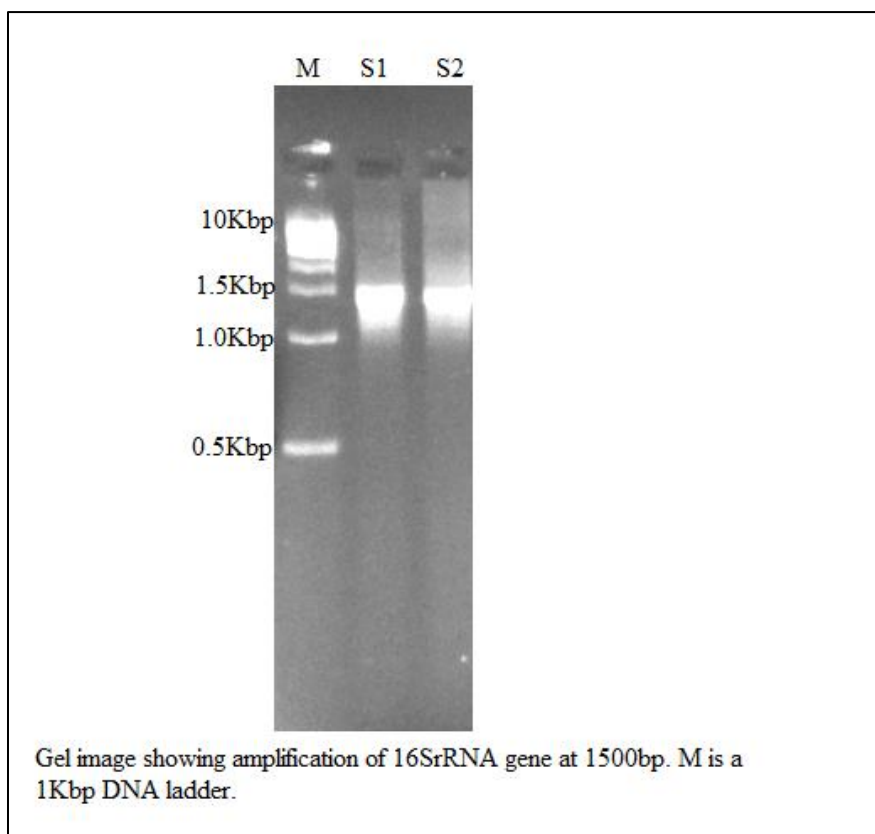


Figure 2

>S2 has 99.62% pairwise similarity with Escherichia coli strain AC1 has NCBI accession number GU594306.1

Table 3: Zones of inhibition of extracts of the leaves *Psidium guajava* on Test bacteria (mm)

Plant extracts	<i>Staph. aureus</i>	<i>Escherichia Coli</i>
Ethanol	10	-
Aqueous	12	-
Control (CIP)	20	10

Note: CIP (Ciprofloxacin for control) 5mg
 Note: the (-) sign indicates no inhibition

Table 4: Zones of inhibition of extracts of the leaves *Dennetia tripetala* on Test bacteria (mm)

Plant extracts	<i>Staph. aureus</i>	<i>Escherichia Coli</i>
Ethanol	-	-
Aqueous	12	10
Control (CIP)	20	10

Note: CIP (Ciprofloxacin for control) 5mg
 Note: the (-) sign indicates no inhibition

Table 5: Minimum inhibitory concentration of the *Dennetia tripetala* leaf extracts on the test bacteria

Test Bacteria	MIC Concentration of leaves extracts of the solvent Extracts (mg/ml)									
	Ethanol					Aqueous extract				
	200	100	50	25	12.5	200	100	50	25	12.5
<i>Staphylococcus aureus</i>	x	x	x	x	x	-	-	+	++	+++
<i>Escherichia Coli</i>	x	x	x	x	x	-	+	+	++	+++

Key: (-) shows no bacteria growth, the solution is clear; (+) shows growth in the solution as turbid; (x) shows no test carried out

Table 6: Minimum inhibitory concentration of the *Psidium guajava* leaf extracts on the test bacteria

Test Bacteria	MIC Concentration of leaves extracts of the solvent Extracts (mg/ml)									
	Ethanol					Aqueous				
	200	100	50	25	12.5	200	100	50	25	12.5
Staphylococcus aureus	-	-	-	-	+	-	-	-	-	+
<i>Escherichia Coli</i>	x	x	x	x	x	x	x	x	x	x

Key: (-) shows no bacteria growth, the solution is clear; (+) shows growth in the solution as turbid; (x) shows no test carried out

DISCUSSION

Plant materials have been determined as major sources of active medicinal substances and antimicrobials. These medicinal plants have been known to have great potentials in combating the numerous diseases and resistant microorganisms [20]. Traditional medicine strategy (2014-2023) was developed to support and strengthen the primary health care role of traditional medicine in keeping the populations healthy. Traditional medicine has continued to play a vital role in treatment and management of disease, especially in primary health care together with the orthodox medicine, especially in developing countries. Therefore medicinal plants are continuously being sourced from the environment and prepared into herbal products. Different techniques are employed in this preparation which include but are not limited to maceration, infusion, decantation, hot steam extraction among others. Antibacterial activity of ethanolic and aqueous extracts of pepper fruit and guava leaves were determined by Agar well diffusion method [21].

This study revealed the presence of many important phytochemicals and secondary metabolites. The results obtained indicated that the leaves of guava plant and pepper fruits contain some major bioactive compounds of known antimicrobial activity such as tannins, saponins, alkaloids, flavonoids, Glycosides and phenols. However, Quinonas was absent in this study as shown in Table 1. This may be attributed to geographical location of the plants, climatic and environmental conditions and impact on the plants. These phytochemicals are all antimicrobial agents. Also, phytochemical studies of ethanolic extract of pepper fruit leaves [21], revealed the presence of antimicrobial agents-tannins, saponins, steroids, flavonoids and phenols. Other researchers, Osuagwu and Eme [22] found similar phytochemicals in their pepper fruit (*D. tripetala*) and guava (*Psidium guajava*) samples [21]. However, both aqueous and ethanolic leaves extracts of guava and pepper fruits lacked the presence of glycoside and Quinonas. The ethanolic extracts of guava leaves did not reveal the presence of saponins. Also, the ethanolic and aqueous extracts of guava (*Psidium guajava*) leaves did not reveal the presence of Glycosides.

The clinical isolation of the Test bacteria *Staphylococcus aureus* and *Escherichia Coli* collected from Annunciation Specialist Hospital Emene were subjected to various biochemical and molecular tests to confirm the identity and also differentiate the Gram positive and Gram negative bacteria as shown in Table 2. The molecular test carried out identified the organisms as having 85% pairwise similarity with *Staphylococcus haemolyticus* strain SH1 (Figure 1) and 99.62% pairwise similarity with *Escherichia coli* strain AC1 respectively (Figure 2).

Antibacterial activity of ethanolic and aqueous extracts of pepper fruit and guava leaves were determined by Agar well diffusion method [21]. The results of the study indicated that both the ethanol and aqueous extracts prepared from the leaves of *Psidium guajava*, showed inhibitory activity against *Staphylococcus aureus* only (Table 3). The Gram-positive bacterium, *Staphylococcus aureus*, was susceptible to the ethanolic and aqueous extracts, at 10mm and 12 mm respectively. While the Gram-negative bacterium, *Escherichia coli*, showed no zone of inhibition on both the ethanolic and aqueous extracts. The resistance of the Gram-negative bacteria could be ascribed to its cell wall structure. Gram-negative bacteria have an active permeability wall, made up of a thin lipopolysaccharide outer membrane, and this could limit the permeation of the extract. Rameshkumar *et al.*, [23] reported that Gram-negative bacteria are usually more resistant to the plant-based antimicrobials and is known to show no effect when compared to Gram-positive bacteria. This is because Gram positive bacteria has a peptidoglycan layer which is easier for permeation of the extracts [24].

Similarly, the findings of this study is in line with that of Hoque *et al.*, [25] in which they found no antibacterial activity of both aqueous and ethanolic extracts of Guava on *E.coli* isolates. Sanches *et al.*, 2005 found that the aqueous extract of guava was effective against *Staphylococcus*. In contrast, the results of Chanda and Kaneria [26], *E.coli* was inhibited by the activity of the different concentrations of Guava ethanol and aqueous leaf extracts. The minimum inhibitory concentration of the extracts revealed that the ethanolic and aqueous extract of the leaf inhibited *Staphylococcus aureus* at

12.5mg/ml. The results of this study indicated that only the aqueous crude solvent extracts prepared from the leaves of *Psidium guajava*, showed the highest inhibitory activity against *Staphylococcus aureus* and no activity on *Escherichia coli* (Table 3).

Additionally the results of the study indicated that the aqueous extracts prepared from the leaves of *Dennettia tripetala* showed inhibitory activity against *Staphylococcus aureus* and *E.coli* (Table 4) with *Staphylococcus aureus* having highest zone of inhibition (20mm). The ethanol extract had no activity on both test microorganisms. The inhibitory activity of *Dennettia tripetala* aqueous leaf extract, is in line with Nwanze *et al.*, [21].

The minimum inhibitory concentration of the extracts revealed that the aqueous extract of *Dennettia tripetala* leaf inhibited *Staphylococcus aureus* and *E.coli* at 50mg/ml and 100 mg/ml (Table 5) respectively while the ethanolic extract of the leaf showed no inhibition to all concentrations. These findings are similar to the observations of Okoh *et al.*, [27]. This could suggest that the aqueous extract of the leaf was more effective at extracting the polar bioactive components [28]. The minimum inhibitory concentration of the extracts equally revealed that both the aqueous and ethanolic extract of *Psidium guajava* leaf inhibited *Staphylococcus aureus* at 12.5mg/ml and 12.5 mg/ml respectively (Table 6).

CONCLUSION

This research demonstrates the antimicrobial potential of *Psidium guajava* and *Dennettia tripetala* leaves extracts by using ethanol and aqueous solvents for extraction. The results of the study, indicate that the aqueous extracts of both plants generally have a better inhibitory property than the ethanolic extract. The results also indicate that the plant ethanolic extracts have no antibacterial effect on the Gram-negative bacteria, showing that they may not contain active ingredients against the organisms. The observed inhibition of Gram-positive bacteria, *Staphylococcus aureus*, suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using ethanol or water as the solvent. On the basis of the present finding, *P. guajava* and *D. tripetala* leaves possess the capabilities of being effective candidates in the search for a natural antimicrobial agent against infections and/or diseases caused by *S. aureus* and *E. coli*.

REFERENCES

1. Fabricant, D., & Farnsworth, N. (2001). The value of plant used in traditional medicine for drug

discovery. *Environmental Health Perspective*, 109(1), 69-75.

2. Moghadamtousi, S. Z., Goh, B. H., Chan, C. K., Shabab, T., & Kadir, H. A. (2013). Biological activities and phytochemicals of *Swietenia macrophylla* King. *Molecules*, 18, 10465-10483.
3. Oyemitan, I. A., Iwalewa, E. O., Akanmu, M. A., & Olugbade, T. A. (2008). Antinociceptive and anti-inflammatory effects of essential oil of *Dennettia tripetala* (G. Baker) (Annonaceae) in rodents. *African Journal of Traditional, Complementary and Alternative Medicines*, 5, 355-360.
4. Iseghohi, S. O. (2015). A review of the uses and medicinal properties of *Dennettia tripetala* (pepperfruit). *Medical Sciences (Basel, Switzerland)*, 3(4), 104-111.
5. Nwachukwu, E. O., & Osuji, J. O. (2008). Evaluation of plant extracts for antifungal activity against *Sclerotium rolfsii* causing cocoyam cormel rot in storage. *Research Journal of Agriculture and Biological Sciences*, 4, 784-787.
6. Begum, S., Hassan, S. I., & Siddiqui, B. S. (2002a). Two new triterpenoids from the leaves from *Psidium guajava*. *Phytochemistry*, 61, 399-403.
7. Prabu, G. R., Gnanamani, A., & Sadulla, S. (2006). Guajaverin a plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. *Journal of Applied Microbiology*, 101, 487-495.
8. Adeyemi, S. O., Akanji, A. M., & Oguntoye, S. A. (2009). Ethanolic leaf extract of *Psidium guajava*: Phytochemical and trypanocidal activity in rats infected with *Trypanosoma brucei brucei*. *Journal of Medicinal Plants Research*, 3(5), 420-423.
9. Belemtougri, R. G., Constantin, B., Cognard, C., Raymond, G., & Sawadogo, L. (2006). Effects of two medicinal plants *Psidium guajava* L. (Myrtaceae) and *Diospyros mespiliformis* L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. *Journal of Zhejiang University Science*, 7, 56-63.
10. Akangi, M. A., Adeyemi, O. S., Oguntoye, S. O., & Sulyman, F. (2009). *Psidium guajava* extract reduces trypanosomiasis associated lipid peroxidation and raises glutathione concentrations in infected animals, *EXCLI Journal*, 8, 148-154.
11. Akharaiyi, F. C., Boboye, B. (2010). Antibacterial and phytochemical evaluation of three medicinal plants. *Journal of Natural Products*, 3, 27-34.
12. Association of Official Analytical Chemists (AOAC). (2016). Official Methods of Analysis of AOAC International - 20th Edition, 2016.
13. Akujobi, C., Anyanwu, B. N., Onyeze, C., & Ibekwe, V. I. (2004). Antibacterial activities and preliminary phytochemical screening of four medicinal plants. *J. Appl. Sci*, 7(3), 4328-4338.

14. Trease, G. E., & Evans, W. C. (1989). *Pharmacognosy*. 11th Edition, Bailliere Tindall, London, 45-50.
15. Harborne, J. B. (1998). *Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*. 5th Edition, Chapman and Hall Ltd, London, 21-72.
16. Nwadioha, S. I., Odimayo, M. S., & Abayomi, F. (2023). *Laboratory Practical Manual for Medical Microbiology and Parasitology Laboratory*, Second Edition, Chapter Five; staining and staining techniques in Microbiology. Chapter Ten, biochemical test for identification of bacteria isolates (pp 40, 54-57).
17. Sagar, A. (2020). *Motility Test- Objectives, Principle, Procedure, Results, Limitation*. New Baneshwor-31, Kathmandu, Nepal.
18. Balouiri, M., Sadiki, M., & Ibensouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*, 6(2), 71-79.
19. National Committee for Clinical Laboratory Standards (NCCLS). (2003). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard: M2-A7*. National Committee for Clinical Laboratory Standards, Wayne.
20. World Health Organization. (2013). *WHO traditional medicine strategy: 2014-2023*. World Health Organization.
21. Nwanze, P., Onyenwuchi, K. S. C., Amaralam, E. C., & Opara, U. L. (2021). Phytochemical Analysis and Antimicrobial Activities of *Dennettia tripetala* Leaf and Seed Extracts Against *Escherichia coli* and *Staphylococcus aureus*. *Journal of Research in Pharmaceutical Science*, 7(10), 05-10.
22. Osuagwu, G. G. E., & Eme, C. F. (2013). The phytochemical composition and antimicrobial activity of *Dennettia tripetala* leaves. *Asian Journal of Natural and Applied Sciences*, 2(3), 69-81.
23. Rameshkumar, K. B., George, V., & Shiburaj, S. (2007). Chemical constituents and antibacterial activity of the leaf oil of *Cinnamomum chemungianum* Mohan et Henry. *Journal of Essential Oil Research*, 19(1), 98-100.
24. Qadan, F., Thewaini, A. J., Ali, D. A., Afifi, R., Elkhawad, A., & Matalka, K. Z. (2005). The antimicrobial activities of *Psidium guajava* and *Juglans regia* leaf extracts to acne developing organisms. *American Chinese Medical*, 33, 197-204.
25. Hoque, M. D. M., Bari, M. L., Inatsu, Y., Juneja, V. K., & Kawamoto, S. (2007). Antibacterial activity of guava (*Psidium guajava* L.) and neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathogens and Disease*, 4(4), 481-488.
26. Chanda, S., & Kaneria, M. (2011) Indian nutraceutical plant leaves as a potential source of natural antimicrobial agents. In: Mendez-Vilas A, editor. *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*, 2, 1251-1259.
27. Okoh, S. O., Iweriegbor, B. C., Okoh, O. O. Nwodo, U. U., & Okoh, A. I. (2016). Bactericidal and antioxidant properties of essential oils from the fruits *Dennettia tripetala* (G. Baker). *BMC Complementary and Alternative Medicine*, 16, 486.
28. Periyar, S. S., Balu, P. M., Kamalraj, S., Raja, V., & Murugesan, S. (2014). Free radical scavenging activity of *Salacia chinensis* root extract in streptozotocin-induced diabetic rats. *Bioresearch Bulletin*, 4(1).