



Phytochemical Analysis and Antimicrobial Activities Of *Dennettia Tripetala* Leaf and Seed Extracts Against *Escherichia coli* AND *Staphylococcus aureus*

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ABSTRACT

The phytochemical and antimicrobial properties of the leaf and seed extracts of *Dennettia tripetala* were studied. Alcohol, cold and hot water solvents were used for the extraction of the bioactive ingredients. According to the results saponins, tannins, phenols, alkaloids, anthranoids, anthraquinones, cardiac glycosides and phlobatannins were observed in the ethanolic extract of the samples while phenols and anthraquinones were not present in either of the aqueous extracts from the leaves. Tannins, phenols, anthraquinones and cardiac glycosides were observed in all the seed extracts. Antimicrobial susceptibility of the plant parts against *Staphylococcus aureus* and *Escherichia coli* was examined with ciprofloxacin as control. The zones of inhibition ranged from 12mm to 18mm for leaf extracts and 10mm to 18mm for seed extracts. The MIC was observed to be 125mg/mL for the ethanolic extracts on *S. aureus* and *E. coli* while it was 250mg/mL for both hot and cold aqueous extracts on both microorganisms. The MIC was observed to be 125mg/mL and 250mg/mL for the ethanolic seed extracts on *S. aureus* and *E. coli* respectively. Both the cold and hot aqueous seed extracts had MIC values of 250mg/mL on both microorganisms. The MBC was 125mg/mL and 250mg/mL for the ethanolic extract on *E. coli* and *S. aureus* respectively. The MBC was 500mg/mL for both the cold and hot aqueous leaf extracts as well as ethanolic seed extracts for both microorganisms while the hot and cold aqueous seed extracts had no bactericidal effect on the test organisms. The results illustrate the need for economic exploitation of these bioactive compounds found in the plant.

Received 13 October, 2021; Revised: 25 October, 2021; Accepted 27 October, 2021 © The author(s) 2021. Published with open access at www.questjournals.org

I. INTRODUCTION

Dennettia tripetala (pepper fruit) belongs to the family Annonaceae. Annonaceae, also called the custard-apple, or Annona family is the largest family of the 4th Magnolia Order (Magnoliales) (Nwachukwu and Osuji, 2008; Iseghohi and Orhue, 2017). According to some authorities, it contains 129 genera and 2,220 species. Many species are valuable for their large pulpy fruits, some are useful for their timber, and others are prized as ornamentals. The family consists of trees, shrubs, and woody climbers found mainly in the tropics, although a few species extend into temperate regions. The leaves and wood are often fragrant. Leaves are simple, with smooth margins, and alternately arranged in two rows along the stems. The radially symmetrical flowers are usually bisexual. In most species the three sepals are united at the base. There are six brown, yellow, or greenish petals, many stamens in a spiral, and many pistils, each with a one-chambered ovary containing many ovules. The fruit is a berry. Flowers in some species are borne directly on large branches or on the trunk (Okwu and Morah, 2004). *Dennettia tripetala* is a medium sized tree found commonly in Carneroun, Ivory Coast, southern, eastern and western areas of Nigeria (Laraetan et al., 2018) and sometimes in Savannah areas (Okwu et al., 2005).

Dennettia tripetala is commonly known as pepper fruit in English, 'mmimi' by the Igbos; 'nkaika' by the Ibibio and Efik; 'omako' by the Urhobo tribe of the Niger-Delta region and 'igberi' by the Yorubas. The bark, leaves, and roots of many species are important in folk medicine, production of perfume and spices (Okolie et al., 2014). The seeds are consumed singly; or taken with kola nut, garden egg or palm wine during cultural entertainment of guests and traditional ceremonies such as weddings and festivals. The seeds are also used to

protect maize grains in storage (particularly effective against *Sitona* spp. and *zeamais*) (Adedire, 2001; Lale, 2002; Nwosu et al., 2017). Many members of the Annonaceae are known to possess various chemical compounds that act as antifeedants, repellants and growth or development inhibitors against many insect species (Anaga et al., 2008; Odeyemi et al., 2008).

The medicinal applications of *Dennettia tripetala* are impressive. Traditional healers use it in conjunction with other therapeutic plants to treat ailments such as convulsions, cough, stomach upset, typhoid, vomiting, edoema, ulcer, diabetes and hypertension (Oyemitan et al., 2006). Phytochemicals contained in *Dennettia tripetala* such as saponins, alkaloids, steroids, flavonoids and terpenoids are thought to be responsible for its medicinal properties (Ejehi and Alcpomedaye, 2005; Laraetan et al., 2018). In addition, its extracts and essential oils possess antifungal, antimicrobial and antiviral properties (Osisiogu, 1975).

II. MATERIALS AND METHODS

Collection and Processing of the Materials

Leaves and seeds of *Dennettia tripetala* were collected from Federal Polytechnic Nekede, Owerri and identified by a botanist. The samples were air-dried and ground into powder using sterile manual grinder. This was stored in air-tight glass containers protected from light and kept until required for analysis.

Preparation of Test Organisms

The clinical isolates of *Staphylococcus aureus* and *Escherichia coli* were collected from the Microbiology Department of Federal Medical Centre, Owerri and identified. The identified isolates were then sub-cultured on sterile nutrient agar. The microbial cultures were diluted with peptone water until the final suspension contained about 1.5×10^8 cfu/mL of *S. aureus* and *Escherichia coli* according to the method of Akujobi et al. (2004). The cell densities obtained were in accordance with 0.5 McFarland's standard and used in all the investigations. The McFarland's standard was prepared by adding 0.1 ml of 1% BaCl₂ into 9.9 ml of 1% sulphuric acid.

Extraction of the Plant Materials

Hot and cold water and alcoholic extraction with ethanol (99%) as described in AOAC (2016) was adopted for this study. Twenty grams (20g) of the grounded sample of *Dennettia tripetala* leaf and seed respectively, were weighed into about 100 ml of water and heated. It was stirred intermittently for 30 minutes and allowed to boil. After boiling, it was filtered using Whatmann's filter paper. For the cold water extraction, the (20g) of the ground samples were placed in 100 ml of distilled cold water and allowed to stay for 4 hours before it was filtered using Whatmann's filter paper. For the ethanolic extraction, 20g of the ground sample was stuffed in a thimble and placed in the extraction chamber of the Soxhlet extractor. Thereafter the Liebig condenser containing the water in-let and outlet hose was fitted into the extraction chamber which was then placed into a flat bottom flask containing 200 ml of ethanol. The apparatus was set up on a heating mantle and then the mantle was connected to the mains. After the extraction, the ethanol was recovered from the mixture using simple evaporation techniques. The alcoholic extract was then used in all the investigations.

Antimicrobial Susceptibility Testing of The Plant Extracts

The disc technique as described by Osadebe and Ukwueze (2004) was adopted for this study to evaluate the antibacterial activity of the extracts. 0.2 ml aliquot of each of the extract was dropped on sterile filter paper discs of 6 millimeters in diameter and allowed to get absorbed before they were placed into nutrient agar plates and appropriately labeled. Discs impregnated with water and ethanol was used as control in each case. The nutrient agar plates were then incubated at 37°C for 24 hours. The zones of inhibitions were measured with a meter rule.

Test for Minimum Inhibitory Concentrations (MIC) of the Plant Extracts

For the MIC test, the plant extract was concentrated by evaporation and two milliliter (2 ml) of the extract was dissolved in four milliliter (4 ml) of peptone water; this gives 500 mg/mL. Thereafter, two fold serial dilutions were carried out from the 500 mg/mL concentration by transferring 2 ml of the 500 mg/mL concentration to 2 ml of peptone water contained in a test tube and homogenizing properly. This procedure of transferring 2 ml of the tube to 2 ml of peptone water contained in the subsequent tubes was continued till the fifth tube. The following concentrations were thereafter obtained: 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL. Having obtained the different concentrations and dilutions, three drops of overnight broth cultures of the test organisms were inoculated into the various dilutions in each case of the test organisms (Akujobi et al., 2004). The tubes were then incubated at 37°C for 24 hours. The lowest concentration of each of the extracts in each case of the extracts (hot, cold and alcoholic extracts) that inhibited the growth of the test organisms was recorded as the MIC. TEST FOR

The Bactericidal/Fungicidal Concentration of the Extract

Tubes showing no visible growth from the MIC test were sub cultured onto sterile nutrient agar plates and incubated at 37°C for 24 hours. The lowest concentration of the extracts yielding no growth was recorded as the Minimum Bactericidal/Fungicidal Concentration as the case may be. PHYTOCHEMICAL SCREENING OF THE EXTRACTS

Test for Saponins

Ten millilitre (10ml) of distilled water was added to two millilitre (2ml) of each of the extracts in a test tube and shaken vigorously (AOAC, 2016). Persistent frothing even after heating is an indication of the presence of saponins.

Test for Anthranoid

The method of Trease and Evans (1989) was adopted for this test. To two millilitre (2ml) of each of the extracts, five millilitre (5ml) of 0.5M potassium hydroxide was added and mixed properly. Then 6 drops of acetic acid was added followed by 2ml of toluene. To the upper layer formed, 2ml of 0.5M potassium hydroxide was added. A change in colour of the mixture is an indication of a positive test while no colour change is an indication of a negative test.

Test for Anthroquinone

To two millilitre (2ml) of each of the extracts, 5ml of 10% ammonia was added and shaken vigorously. 2ml of benzene was thereafter added. A colour change is an indication of a positive test while none is an indication of a negative test (Trease and Evans, 1989).

Test for Phenol

The method of Harbome (1973) was employed. 5ml of each of the extracts was mixed with 8ml of distilled water in a test tube and 6ml of ferric chloride was added to the mixture. A colour change to light brown is an indication of a positive test while none indicates a negative test.

Test for Alkaloid

To two millilitre (2ml) of each of the extracts, 5ml of 1% aqueous hydrochloric acid was added and placed in a water bath for 3 minutes and thereafter 3 drops of Mayer's reagent was added (Trease and Evans, 1989). A white precipitate is an indication of a positive test while none indicates a negative test.

Test for Tannins

The method of Harbome (1973) was employed. To one millilitre (1ml) of each of the extracts, 2ml of 1% ferric chloride was added. A colour change is an indication of a positive test while none is an indication of a negative test.

Test for Phlobatannins

To two millilitre (2ml) of each of the extracts, 1% aqueous hydrochloric acid added and boiled. The presence of white precipitate is an indication of a positive test while none is an indication of a negative test (Trease and Evans, 1989).

Test for Cardiac Glycoside

The Salkowski test was employed in this test. To one millilitre (1ml) of each of the extracts, 2ml of chloroform was added and then 2ml of concentrated tetraoxosulphate (VI) acid was added to form a lower layer. A reddish brown colour at the inter phase is an indication of a positive test while none is an indication of a negative test.

III. RESULTS

Table 4.1 represents the phytochemical constituents of the plant extracts. According to the results, saponins, tannins, phenols, alkaloids, anthranoid anthraquinones, cardiac glycosides and phlobatannins were all observed in the ethanolic extract of the samples while phenols and anthraquinones were not present in either of the aqueous extracts from the leaves. Saponins, alkaloids, and phlobatannins were not observed in any of the seed extracts. However, tannin, phenols, anthraquinones and cardiac glycosides were observed in all the seed extracts.

Table 4.2 shows the results for the antimicrobial susceptibility of the plant part against *Staphylococcus aureus* and *Escherichia coli*. The zones of inhibition recorded ranged from 12mm to 18mm for leaf extracts while seed extract produced zones of inhibition ranging from 10mm to 18mm. The ethanol extracts produced higher zones of inhibition followed by the hot and cold aqueous extracts, respectively.

Table 4.3 shows the results for the minimum inhibitory concentration of the plant extracts. The MIC was observed to be 125mg/mL for the ethanolic extracts on *S. aureus* and *E. coli* while it was 250mg/mL for both hot and cold aqueous extracts on both microorganisms respectively. The MIC was observed to be 125mg/mL and 250mg/mL for the ethanolic seed extracts on *S. aureus* and *E. coli* respectively. Both the cold and hot aqueous seed extracts had MIC values of 250mg/mL on both microorganisms.

Table 4.4 shows the result for the minimum bactericidal concentration of the plant part extracts. According to the results, the MDC was 125mg/mL and 250mg/mL for the ethanolic extract on *E. coli* and *S. aureus* respectively. The MDC was 500mg/mL for both the cold and hot aqueous leaf extracts for both

microorganisms. 500mg/mL was the MBC for the seed ethanolic extract on *E. coli* and *S.aureus* while the hot and cold aqueous seed extracts had no bactericidal effect on the test organisms.

Table 4.1: Photochemical constituents of the plant extracts

Photochemical	PLAINT PART EXTRACTS					
	Leaf			seed		
	E	H	C	E	H	C
Saponins	+	+	+	-	-	-
Tannins	+	+	+	-	-	-
Phenols	+	+	+	-	-	-
Alkaloids	+	+	+	-	-	-
Anthranoids	+	+	+	-	-	-
Anthraquinones	+	+	+	-	-	-
Cardiac glycosides	+	+	+	-	-	-
Phylobatannins	+	+	+	-	-	-

Keys:- - = Absence of phytochemicals
 water extract + = Presence of phytochemicals E = Ethanol extracts, C = Cold

Table 4.2: Zones of inhibition of the plants extracts (mm) on test organisms

Test organisms (mm)	Diameter of plants part zones of inhibition											
	R			I			S			disk content		
	Leaf			Seed			CP			disk content		
Staphylococcus aureus	18 12 14			18 12 14 28			100ug					
≤20	21-28			≥29								
Escherichia coli	18 12 14			16 10 12 26			100ug					

Keys:- E = ethanol extract, R = Resistance, H = hot water extract, I = Intermediate, C = Cold water extracts
 Susceptible, CP = Ciprofloxacin, CLSI = Clinical and Laboratory Standard Institute, Mm = millimeter.

Table 4.3: Minimum inhibitory concentration of the plant part extracts

Test organisms	Plants part/concentration (mg/mL)																						
	H				E				C				H				seed						
	E				C				H				E				C						
500 250 125 62.5	500 250 125 62.5				500 250 125 62.5				500 250 125 62.5				500 250 125 62.5				500 250 125 62.5						
Staphylococcus	-	-	-	+	-	-	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
aureus	18 12 14				18 12 14 28				100ug														
≤20	21-28				≥29																		
Escherichia coli	18 12 14				16 10 12 26				100ug														

IV. DISCUSSION

The results of the phytochemical screening revealed the presence of saponins, tannins, phenols, alkaloids, anthranoids, anthraquinones, cardiac glycoside and phylobatannins in the ethanolic extract of the leaves of *Dennattia tripetala*. It recorded the highest member of phyto chemicals in comparison with the other extracts. Saponin was absent in the seed extracts. All the plant extracts had tannins and anthroquinone. Osuagwu and Eme (2013), Laraetan et al, (2018) and Omega et al. (2018) found similar abundance of phyto chemicals in

their *D. tripetala* samples. These phytochemicals no doubt were responsible for the antimicrobial properties of the plant extracts (Osugwu et al., 2007).

The ethanolic extract of the leaf had zones of inhibition of 18mm each against *Staphylococcus aureus* and *Escherichia coli* followed by the hot water extracts which had 14mm each and the cold extracts had zone of inhibition of 12mm each. Comparatively, the zones of inhibition of the leaf extracts were slightly higher than those of the seed extracts.

Osugwu and Eme (2013) reported the antimicrobial properties of *Dennettia tripetala*. The zones of inhibition ranged from 8mm to 14mm. Ogbonna et al. (2013) also reported the antimicrobial activities of the extract of *Dennettia tripetala* seed on *Escherichia coli*. In the study, hot water, cold water and ethanolic extracts of the seed were used. The result showed that the 50 microgram per milliliter concentration of the seed extracts had zones of inhibitions of 5.0mm (cold water), 7.5mm (hot water) and 11mm (alcoholic extract), a trend that was also observed in the present study along with Okoh et al. (2016). This shows that the extracts of *Dennettia tripetala* can be used in the development of novel antibiotics against the test microorganisms (Ejehi and Akpomedaye, 2005; Nwogu et al., 2008; Okoh et al., 2016).

The minimum inhibitory concentration of the extracts revealed that the ethanolic extract of the leaf inhibited both bacteria at 250mg/ml while the ethanolic extract of the seed inhibited it at 500mg/ml. The findings are similar to the observations of Okoh et al. (2016) though inhibition was seen at lower MIC values (20 mg/ml). This suggests that the ethanolic extract of the leaf was more effective at extracting the polar bioactive components or better yet, that the components were more prevalent in the leaves (Chavan et al., 2013; Periyar et al., 2014).

The MBC was 125mg/ml and 250mg/ml for the ethanolic extract of *E. coli* and *S. aureus* respectively. The MBC was 500mg/ml, for both the cold and hot aqueous leaf extracts for both microorganisms. 500mg/ml was the MBC for the seed ethanolic extract on *E. coli* and *S. aureus* while the hot and cold aqueous seed extracts had no bactericidal effect on the test organisms. It is evident that *Dennettia tripetala* has antimicrobial properties (Anyale and Amusan, 2003; Soetan et al., 2009; Isieghohi, 2015). The various phytochemicals may be responsible for its pharmacological activities. Tannins have a broad spectrum of action against pathogenic fungi, viruses and bacteria and act as antibiotics (Proestos et al., 2013; Luractan et al., 2018). Alkaloids are used as analgesic, antibiotics, and antispasmodics (Omage et al., 2018) while saponins serve as emulsifiers and antifungal agents.

V. CONCLUSION

The outcome of this work has shown that the extracts of *Dennettia tripetala* exhibited zones of inhibition against *Staphylococcus aureus* and *Escherichia coli* and thus has the potentials of being used in the production of drugs against these organisms.

VI. RECOMMENDATIONS

- The extract should be used for antibiotic production to curb the rising growth of drug resistant pathogens.
- The extracts of pepper fruit (*Dennettia tripetala*) may be purified and developed into drugs since purifying these extracts may increase its antimicrobial property.
- Attention should be given to herbal medicine practitioners who claim that herbal products have cure to some diseases by scientifically testing their products.

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