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Cola Acuminata Nut Show Indication of Arrhythmia Management: Laboratory Examination of Extracts of Raw Versus Heat Processed Nuts

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Abstract

Chewing of nuts of *Cola acuminata* is a cultural practice in any African countries especially Ghana and Nigeria. The current study investigated antibacterial activity and phytochemical composition of methanol and ethanol Soxhlet extracts of raw, steamed and roasted cola nut on *Pseudomonas aeruginosa* and *Staphylococcus aureus* using the agar well diffusion method. Amoxillin and distilled water were used as controls. The ranges of mean zones of inhibition of the methanol and ethanol extracts of raw cola nut at 200mg/ml tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 10.00±0.00 to 15.00±0.00 and 10.50±1.50 to 12.00±0.00 respectively. The ranges of the mean zones of inhibition of the methanol and ethanol extracts of steamed cola nut at 200mg/ml tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 8.00±0.00 to 7.00±0.00 and 7.50±0.50 to 8.50±0.50 respectively. Those of the methanol and ethanol extracts against *Pseudomonas aeruginosa* and *Staphylococcus aureus* were 6.00±0.00 to 7.00±0.00 and 5.00±0.00 to 6.00±1.00 respectively. Raw cola nut was found to be most effective against the two microbes. However, Amoxicillin at concentration 200mg/ml presented the highest mean zones of inhibition against *Staphylococcus aureus* (36.00±0.00) and *Pseudomonas aeruginosa* (29.00±0.00), while distilled water had no inhibitory on the two microbes. Extract concentrations from 120–30 mg/ml had similar trends of inhibition with the 200mg/ml concentration. Phytochemical screening revealed the presence of saponins, terpenoids, alkaloids, tannins, flavonoids and phenols in both methanol and ethanol extracts of the raw, steamed and roasted cola nut.

Keywords: *Cola acuminate*; Antimicrobial; *Staphylococcus aureus*; *Pseudomonas aeruginosa*.

1. Introduction

There is a worldwide effort to find agents that can encourage healing and thus decrease hospitalization costs and save patients from very serious health problems [1] and it has been reported that medicinal plants products could be used widely used [2]. Plants are a source of medicine for man. Traditionally, plants have been used in the management and prevention various diseases ranging from colds and coughs to inflammations and infections mainly due to presence of phytochemicals [3, 4]. The use of medicinal plants has been substantially endorsed for primary health care throughout the world, particularly in South Saharan African nations [5].

Hypocholesterolaemic, immunostimulant, hypoglycemic and anticarcinogenic characteristics of phytochemicals are known. Tannins are polyphenols extracted from multiple species abundantly in the bark, wood, fruit, fruit pod, leaves and roots [6]. All forms of tannins may be involved in blood glucose level management. Tannin was shown to stimulate carbohydrate use in the receptor cells [7], and anti-diabetic activity. Alkaloids are also known to have reduced activity in blood glucose. It has been indicated that alkaloids tetrandine and berberine are accountable for multiple biological activities [7].

Cola nut is the fruit of the kola tree of the family Sterculiaceae, The most commonly used are *C. verticillata, C. acuminata and C. nitida*, with the latter two having the greatest economic importance. Cola nut is a native stimulant which commonly chewed in its raw form in many West African cultures. It is often used ceremonially and to honour guest [8]. The nuts are reported to contain about 2-4% caffeine and threobromine as well as tannins, alkaloids, saponins and flavonoids. It is used as a flavouring ingredient in beverages, in folk medicine, many herbal formulations and has potential therapeutic benefits due largely to the activity of their flavonoids and other bioactive compounds [9]. It has been used as an aphrodisiac, appetite suppressant and also to treat throat infection, cough and stomach upset [10].

Primary classes of antimicrobial agents such as disinfectants are "non-selective antimicrobials" kill a broad variety of microbes on non-living surfaces to avoid disease spread, antiseptics and antibiotics [11]. Antimicrobial and antioxidant activities have also been discovered in compounds such as terpenoids, flavonoids, polyphenols,

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alkaloids, tannins, saponins, pigments, enzymes and minerals [12]. Some of these compounds have been reported in cola nuts, making it a possible efficient product in the treatment of bacterial infections [10]. However, most plant extracts are used without standardization to treat illnesses [13].

We sought the study the potential of cola nuts to aid in maintenance of health. In the current study extracts of differently treated cola nuts were used in phytochemical and antimicrobial studies. Studies were done for alkaloids, saponins, flavonoids, phenols, terpenoids, and tannins. Antimicrobial activity of nut extracts and a standard drug on and *Pseudomonas aeruginosa* and *Staphylococcus aureus* was also evaluated and compared. The Minimum Inhibition Concentration (MIC) ranges of the extracts were also determined.

2. Materials

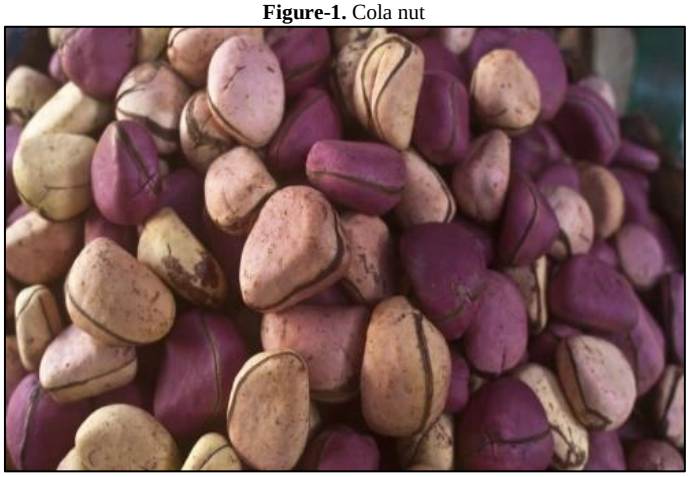
Cola nuts, microbes (*Staphylococcus aureus* and *Pseudomonas aeruginosa*), reagents, Mueller-Hinton agar and various equipment were used.

3. Methods

3.1. Sample Collection and Preparation

The cola nuts were purchased from Central Market in Navrongo, in the Upper East Region of Ghana. Sample authentication was done at the Department of Applied Biology of the University for Development Studies (UDS). *Pseudomonas aeruginosa* and *Staphylococcus aureus* used in the study were obtained from the microbiology laboratory of the Department of Applied Biology, UDS.

Figure-1. Cola nut



The cola nuts were treated in three different ways before the extraction:

- One portion of the nuts was steamed over boiling water at a temperature of 100ºC in a water bath.
- A second portion of the cola nuts was roasted over direct heat of a heating mantle.
- The third portion was used raw.

All the samples were dried and powdered using a mechanical grinder.

3.2. Preparation of Extracts

The reflux extraction method was used.

After the reflux apparatus was set up, about 50g of the powdered cola nut was placed in a thimble and put into the three headed round bottomed flask. The solvents used for extraction were ethanol and methanol separately.

Dry weight of the extract was obtained after solvent evaporation, after which aqueous solutions of the extracts were used for the antimicrobial analysis. The extracts were prepared in concentrations of 200, 120, 60 and 30 mg/ml for antimicrobial studies.

3.3. Microbial Preparation

Colonies of the clinical isolates were transferred with a sterile loop into a tube containing 5 ml of normal saline and incubated at 37 °C for 24 hours.

3.4. Antibacterial Activity Tests

The agar preparation was carried out following the method described by Abubakar, *et al.* [3]. 27g of Mueller-Hinton agar was weighed into a 2 litre beaker and topped up with 720 ml of distilled water. The resultant solution was stirred to form a uniform clear solution and then heated to boil on a hot plate. The solution was then kept in an autoclave at 121 °C for 45 minutes to ensure maximum sterility.

3.5. Sensitivity Test

The sterile solution was allowed to cool to a temperature of about 60 °C and then poured into culture dishes (15-20 ml). The test organisms were spread on solidified agar and wells (7 mm) created with a cork borer and filled with

about 0.2 ml of various concentrations (200, 120, 60 and 30mg/ml) of plant extracts. Amoxicillin and distilled water were used as positive and negative controls respectively. Each concentration of extract was duplicated for each strain and left to stand for two hours for the extracts and the control to diffuse into the media before it was placed in the incubator at 37 °C for 24 hours. The relative susceptibility of the strains (organisms) to the extracts was indicated by clear zone of inhibition produced after inoculation. It was measured using a divider and a ruler and was recorded in millimetre [14].

3.6. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of the extracts was carried out using the agar well diffusion method. Further variable concentrations of the extract was prepared by serial dilution to obtain concentrations of 200, 120, 60 and 30mg/ml. Molten Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer and poured aseptically into sterile petri dishes to a depth of 4 mm. Care was taken while pouring the media into the plate in order to obtain a uniform depth and surface and allowed to solidify. Test organisms in normal saline were spread on the solidified agar. Wells (7 mm depth) were created with a sterile cork-borer at wide enough intervals and filled with the various concentrations of the plant extracts. Same quantity of the negative control (distilled water) and positive control (amoxicillin) was drawn into a well each on the same plate. Plates were left to stand for about 30 minutes until complete diffusion into the medium at room temperature and finally incubated in inverted positions at 37 °C for 24 h. Tests were carried out in duplicates after which they were observed for minimum inhibitory concentrations for the unformulated extract. The process was repeated and the wells filled with the extract to obtain the minimum inhibitory concentrations for the formulated extract. The lowest concentration of extract showing a zone of inhibition was taken as MIC [15].

3.7. Phytochemistry

Qualitative detection of active phytochemical constituents of the extracts was carried out for all using the standard procedures [16].

4. Results

4.1. Physical Properties of the Extracts

The six extracts were identical because they were all derived from cola nuts. Both the ethanol and methanol extracts of raw nuts were brownish orange in colour, and both the ethanol and methanol extracts of the steamed nuts were also reddish brown in colour. Both the ethanol and methanol extracts of the roasted nuts were dark brown in colour.

4.2. Phytochemistry

Table-1. Phytochemical makeup of raw and treated cola nuts

Extract		Saponins	Alkaloid	Tannins	Terpenoids	Flavonoids	Phenols
Raw Cola nut	Ethanol	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+
Roasted Cola nut	Ethanol	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+
Steamed Cola nut	Ethanol	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+

"+" = present; " – " = absent

4.3. Antimicrobial Activity

Zones of inhibition of Staphylococcus aureus and Pseudomonas aeruginosa)

i. Extracts of raw cola nuts

ii. Extracts of steamed cola nuts

iii. Extracts of roasted cola nuts

Table-2a. Methanol Extract on *Staphylococcus aureus and Pseudomonas aeruginosa*

Conc (mg/ml)	Mean ± SD (mm)					
	Staphylococcus aureus			*Pseudomonas aeruginosa*		
	Extract	Amoxicillin	Distilled water	Extract	Amoxicillin	Distilled water
200.00	10.00 ± 0.00	36.00± 0.00	0.00 ± 0.00	15.00 ± 0.00	29.00± 0.00	0.00 ± 0.00
120.00	10.50 ± 0.50	29.00 ±0.00	0.00 ± 0.00	7.50 ± 2.50	23.00± 0.00	0.00 ± 0.00
60.00	2.50 ± 0.50	21.00 ±0.00	0.00 ± 0.00	5.50 ± 1.50	19.00± 0.00	0.00 ± 0.00
30.00	0.00 ± 0.00	16.00 ±0.00	0.00 ± 0.00	2.00 ± 1.00	11.00± 0.00	0.00 ± 0.00

Table-2b. Ethanol Extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Conc (mg/ml)	Mean ± SD (mm)					
	Staphylococcus aureus			*Pseudomonas aeruginosa*		
	Extract	Amoxicillin	Distilled water	Extract	Amoxicillin	Distilled water

200.00	10.50 ± 1.50	36.00± 0.00	0.00 ± 0.00	12.00 ± 0.00	29.00± 0.00	0.00 ± 0.00
120.00	10.00 ± 1.00	29.00 ±0.00	0.00 ± 0.00	12.00 ± 1.00	23.00 ±00.0	0.00 ± 0.00
60.00	1.50 ± 0.50	21.00 ±0.00	0.00 ± 0.00	6.00 ± 1.00	19.00 ±0.00	0.00 ± 0.00
30.00	0.00 ± 0.00	16.00 ±0.00	0.00 ± 0.00	1.50 ± 0.50	11.00 ±0.00	0.0 0.00

ii. Extracts of steamed cola nuts

Table-3a. Methanol Extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Conc (mg/ml)	**Mean ± SD (mm)**					
	Staphylococcus aureus			***Pseudomonas aeruginosa***		
	Extract	**Amoxicillin**	**Distilled water**	**Extract**	**Amoxicillin**	**Distilled water**
200.00	8.00 ± 0.00	36.00±0.00	0.00 ± 0.00	7.00 ± 0.00	29.00± 0.00	0.00 ± 0.00
120.00	8.00 ± 1.00	29.00 ±0.00	0.00 ± 0.00	4.50 ± 0.50	23.00 ±0.00	0.00 ± 0.00
60.00	3.00 ± 0.00	21.00 ±0.00	0.00 ± 0.00	2.00 ± 1.00	19.00 ±0.00	0.00 ± 0.00
30.00	0.00 ± 0.00	16.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ±0.00	0.00 ± 0.00

Table-3b. Ethanol Extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Conc (mg/ml)	**Mean ± SD (mm)**					
	Staphylococcus aureus			***Pseudomonas aeruginosa***		
	Extract	**Amoxicillin**	**Distilled water**	**Extract**	**Amoxicillin**	**Distilled water**
200.00	7.50 ± 0.50	36.00± 0.00	0.00 ± 0.00	8.50 ± 0.50	29.00 ± 0.00	0.00 ± 0.00
120.00	6.50 ± 1.50	29.00 ± 0.0	0.00 ± 0.00	4.00 ± 1.00	23.00 ± 0.00	0.00 ± 0.00
60.00	0.00 ± 0.00	21.00 ±0.00	0.00 ± 0.00	1.50 ± 0.50	19.00 ± 0.00	0.00 ± 0.00
30.00	0.00 ± 0.00	16.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 0.00	0.0 0.0 0

iii. Extracts of roasted cola nuts

Table-4a. Methanol extract on Staphylococcus aureus and Pseudomonas aeruginosa

Conc (mg/ml)	**Mean ± SD (mm)**					
	Staphylococcus aureus			***Pseudomonas aeruginosa***		
	Extract	**Amoxicillin**	**Distilled water**	**Extract**	**Amoxicillin**	**Distilled water**
200.00	7.00 ± 0.00	36.00± 0.00	0.00 ± 0.00	6.00 ± 0.00	29.00± 0.00	0.00 ± 0.00
120.00	3.50 ± 0.50	29.00 ±0.00	0.00 ± 0.00	3.00 ± 1.00	23.00 ± 0.00	0.00 ± 0.00
60.00	1.50 ± 0.50	21.00 ±0.00	0.00 ± 0.00	2.00 ± 1.00	19.00 ± 0.00	0.00 ± 0.00
30.00	0.00 ± 0.00	16.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 0.00	0.00 ± 0.00

Table-4b. Ethanol extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Conc (mg/ml)	**Mean ± SD (mm)**					
	Staphylococcus aureus			***Pseudomonas aeruginosa***		
	Extract	**Amoxicillin**	**Distilled water**	**Extract**	**Amoxicillin**	**Distilled water**
200.00	6.00 ± 0.00	36.00± 0.00	0.00 ± 0.00	5.00 ± 1.00	29.00± 0.00	0.00 ± 0.00
120.00	2.00 ± 1.00	29.00 ±0.00	0.00 ± 0.00	4.50 ± 0.50	23.00 ± 0.00	0.00 ± 0.00
60.00	0.00 ± 0.00	21.00 ±0.00	0.00 ± 0.00	1.00 ±0.00	19.00 ± 0.00	0.00 ± 0.00
30.00	0.00 ± 0.00	16.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 0.00	0.00 ± 0.00

4.4. Minimum Inhibitory Concentration Ranges

The minimum concentrations of the methanol and ethanol extracts able to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were also determined as shown in table 5 below.

Table-5. Minimum Inhibitory Concentration Ranges of Extracts

Sample	**Extract**	**MIC (mg/ml)**	
		Staphylococcus aureus	***Pseudomonas aeruginosa***
Raw Cola nut	Methanol	60 - 30	30 – 15
	Ethanol	60 – 30	30 – 15
Steamed Cola nut	Methanol	60 – 30	60 - 30
	Ethanol	100 – 80	60 - 30
Roasted Cola nut	Methanol	60 – 30	60 - 30
	Ethanol	120 – 100	60 - 30
Control	Amoxicillin	10.50	10.50

5. Discussion

Secondary metabolites are used extensively in veterinary medicine, agriculture, pharmaceutical, human therapy and scientific research industries [17]. A successful determination of the various phytochemicals in plants is mainly dependent on the type of solvent used in the extraction process [18].

The new generation of organic antibiotics has certain disadvantages such as high cost of drugs and adverse side effects as well as resistance of microbes to these antibiotics which has resulted in increased morbidity and mortality. Hence the need to explore for substances from other sources with established antimicrobial activity. Consequently, this has led to the search for more valuable antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as sources and templates for the synthesis of new antimicrobial drug [19].

Current results show that the methanol and ethanol extracts of the three samples of cola nut contained some phytochemicals. Alkaloids, saponins, terpenoids, tannins and phenols were present in each of the samples (Table 4). Flavonoids, alkaloids, and saponins have been investigated and proven to possess anti-inflammatory and anti-cancer properties. The presence of these secondary metabolites justifies the use of cola nut as an antimicrobial agent.

It was observed generally that both the ethanol and methanol extracts of the three samples of cola nut showed inhibitory effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Terpenoids and alkaloids have been known to exhibit antimicrobial activity. The mechanism of action alkaloids is attributed to their ability to intercalate with DNA [20].

There is therefore a reason to believe that the inhibitory activity of the cola nuts could be attributed to the presence of terpenoids and alkaloids as well as saponins and flavonoids as found in the preliminary qualitative phytochemical analysis (Table 1).

The results of the inhibitory effects of each of the extracts at different concentrations were expressed as Mean±SD of two replicates. The mean zone of inhibition in application of 200mg/ml of the methanol extract of raw cola nut was in the order 15.00±0.00mm *(P. aeruginosa*) > 10.00±0.00mm (*S. aureus*). Decreasing the concentration to 120mg/ml, the order of mean zone of inhibition changed with a decrease in the zone of inhibition of *P. aeruginosa* and an increase in the zone of inhibition of *S. aureus*; 7.50±2.50mm *(P. aeruginosa)* < 10.50±0.50mm (*S. aureus*). But decreasing the concentration to 60mg/ml, the zone of inhibition of *P. aeruginosa* increased while the zone of inhibition of *S. aureus* decreased; 5.50±1.50mm (*P. aeruginosa*) > 2.50±0.50mm (*S. aureus)*. A further decrease in the concentration to 30mg/ml showed the mean zone of inhibition in the order; 2.00±1.00 mm (*P. aeruginosa*) and no zone of inhibition of *S. aureus.* These observations clearly show that the methanol extract of raw cola nut has a higher inhibitory effect on *P. aeruginosa* compared to *S. aureus.*

The mean zone of inhibition of 200mg/ml of the ethanol extract of raw cola nut was also in the order of 13.00±1.00mm (*P. aeruginosa*) < 10.50±1.50mm (*S. aureus*). Decreasing the concentration to 120mg/ml, the order of mean inhibition was maintained but with a decrease in the Mean±SD in both microbes; 12.00±1.00mm (*P. aeruginosa*) > 10.00±1.00mm (*S. aureus*). At concentrations 60mg/ml and 30mg/ml, the mean zones of inhibitions were observed in the order of 6.00 ± 1.00mm (*P. aeruginosa*) > 1.50±0.50mm (*S. aureus*) and 1.50±0.50mm (*P. aeruginosa*) and no inhibition for *S. aureus* respectively. These observations clearly show that the ethanol extract of raw cola nut has a higher inhibitory effect on *P. aeruginosa* than on *S. aureus*.

Also comparing the inhibitory activity of the methanol extract of raw cola nut to that of the ethanol extract, it was observed that at 200mg/ml, the mean zone of inhibition of the methanol extract was 15.00±0.00mm *(P. aeruginosa*) and 10.00±0.00mm (*S. aureus*) while that of the ethanol was 13.00±1.00mm (*P. aeruginosa*) >10.50±1.50mm (*S. aureus*). At 120mg/ml, the mean zone of inhibition of the methanol extract was 7.50±2.50mm (*P. aeruginosa*) and 10.50±0.50mm (*S. aureus*) whiles that of the ethanol extract was 9.00±1.00mm (*P. aeruginosa*) and 10.00±1.00mm (*S. aureus*). Decreasing the concentration to 60mg/ml, the mean zone of inhibition of the methanol extract was 5.50±1.50mm (*P. aeruginosa*) > 2.50± 0.50mm (*S. aureus)* whereas the ethanol was 4.00±1.00mm (*P. aeruginosa*) > 1.50±0.50mm (*S. aureus*). These results show that both extracts have higher inhibitory activity on *P. aeruginosa* compared to that of *S. aureus*. Also, the methanol extracts showed higher inhibitory activity on the two microbes compared to the ethanol extract.

Also the zone of inhibition of methanol extract of steamed cola nut was at 200mg/ml in the order of 7.00±0.00mm (*P. aeruginosa*) < 8.00±00mm (*S. aureus*). At 120mg/ml and 60mg/ml, the mean zones of inhibition were observed in the order of 4.50±0.50mm (*P. aeruginosa*) < 8.00±1.00mm (*S. aureus*) and 2.00±0.00mm (*P. aeruginosa*) < 3.00±0.00mm *(S. aureus*) respectively. These observations show that the methanol extract of steamed cola nut has a greater inhibitory activity on *S. aureus* compared to *P. aeruginosa.*

The ethanol extract of steamed cola nut at 200mg/ml had mean zones inhibition in an order of 8.50±0.50mm (*P. aeruginosa*) > 6.50±1.50mm (*S. aureus*). Decreasing the concentration to 120mg/ml, the mean zone of inhibition was observed in this order; 4.00±1.00mm *(P. aeruginosa*) < 7.50±0.50mm (*S. aureus*). From these results, it can be observed that the ethanol extract of steamed cola nut has a higher inhibitory activity on *S. aureus* compared to *P. aeruginosa.*

Comparing the mean zones of inhibition of the methanol and ethanol extracts of steamed cola nut, the mean zone of inhibition of the methanol extract at 200mg/ml was 7.00±0.00mm (*P. aeruginosa*) and 8.00±00mm (*S. aureus*) whiles that of the ethanol extract was 8.50±0.50mm (*P. aeruginosa*) and 6.50±1.50mm (*S. aureus*). At 120mg/ml, the mean zone of inhibition of the methanol extract was 4.50±0.50mm (*P. aeruginosa*) < 8.00±1.00mm (*S. aureus*) whiles that of the ethanol extract was 4.00±1.00mm *(P. aeruginosa*) < 7.50±0.50mm (*S. aureus*). The comparisons indicate that the ethanol extracts of steamed cola nut has a higher inhibitory activity on *P. aeruginosa* compared to *S. aureus* whiles the methanol extract has a higher inhibitory activity on *S. aureus* compared to *P.*

aeruginosa. However, the zones of inhibition shown by the methanol extracts are higher compared to that of the ethanol extracts.

The mean zone of inhibition of methanol extract of roasted cola nut at 200mg/ml was in the order; 6.00 ± 0.00mm (*P. aeruginosa*) < 7.00±0.00mm *(S. aureus*). Decreasing the concentration to 120mg/ml and 60mg/ml the mean zone of inhibition was in the order; 3.00±1.00mm (*P. aeruginosa*) < 3.50±0.50mm (*S. aureus*) and 1.00±0.00mm (*P. aeruginosa*) < 1.50±0.50mm (*S. aureus*) respectively. These observations show that the methanol extract of roasted cola nut has a higher inhibitory activity on *S. aureus* compared to *P. aeruginosa.*

Also, the mean zone of inhibition of ethanol extract of roasted cola nut at 200mg/ml was 5.00±1.00mm (*P. aeruginosa*) < 6.00±2.00mm (*S. aureus*).Decreasing the concentration to 120mg/ml and 60mg/ml, the mean zone of inhibition was 4.50±0.50mm (*P. aeruginosa*) > 2.00±1.00mm (*S. aureus*) and 1.00±0.00mm (*P. aeruginosa*) and no zone of inhibition for *S. aureus*. From the results, the ethanol extract of roasted cola nut has a higher inhibitory activity on *P. aeruginosa* compared to *S. aureus*.

Comparing the mean zone of inhibition of the methanol and ethanol extract of the roasted cola nut, the mean zone of inhibition of the methanol extract at 200mg/ml was 6.00±0.00mm (*P. aeruginosa*) and 7.00±0.00mm *(S. aureus*) while that of ethanol was 5.00±1.00mm (*P. aeruginosa*) and 6.00±2.00mm (*S. aureus*). At 120mg/ml, the mean inhibition of the methanol extract was 3.00±1.00mm (*P. aeruginosa*) and 3.50±0.50mm (*S. aureus*) whiles that of ethanol was 4.50±0.50mm (*P. aeruginosa*) and 2.00±1.00mm (*S. aureus*). From the results, it can be observed that the methanol extract has a higher inhibitory activity on *S. aureus* compared to *P. aeruginosa* and the ethanol extract has a higher inhibitory activity on *P. aeruginosa* compared to *S. aureus*. However, the zones of inhibition shown by the methanol extracts.

Comparing the inhibitory activity of the raw, steamed and roasted cola nut extracts, it was observed that at 200mg/ml of the extracts, raw cola nut methanol extract had the higher mean zone of inhibition (15.00±0.00 mm) on *P. aeruginosa.* The second highest mean zone of inhibition was constituted by ethanol extract of raw cola nut on *S. aureus.* The lowest mean of inhibition at the same concentration was found in ethanol extract of roasted cola nut (5.00±1.00) on *P. aeruginosa.*

The mean zones of inhibition of the extracts were also compared to that of amoxicillin which was used as positive control for the bacteria and distilled water which was used as negative control. From the results, it can be observed that at 200mg/ml, the standard (amoxicillin) presented the highest inhibitory effect against *Staphylococcus aureus* (36.00±0.00 mm) and *Pseudomonas aeruginosa* (29.00±0.00 mm), while the distilled water showed no inhibitory effect on the two microbes. This shows that the inhibitory effects that were observed were solely due to the active ingredients present in the extract with no contribution from the solvent.

Indabawa and Arzai [21], researched on the raw cola nut against selected human pathogens also revealed that the methanol extract had inhibitory activity on *Staphylococcus aureus*.

The Minimum Inhibitory Concentrations (MIC) of the extracts of the two plants were also evaluated. It was observed that each of the extracts exhibited varying MIC against the *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The minimum concentration of the methanol extract of the raw cola nut that impeded the growth of the microbes was observed between 30 - 15mg/ml (*Pseudomonas aeruginosa*) and between 60 -30mg/ml (*Staphylococcus aureus*) whereas that of the ethanol extract was 30 -15mg/ml (*Pseudomonas aeruginosa*) and between 60 - 30mg/ml (*Staphylococcus aureus*).These observations show clearly that the methanol and ethanol showed similar inhibitory effects at lower concentrations on *Pseudomonas aeruginosa*, each with MIC between 30 – 15mg/ml. However, inhibition of the extracts on *Staphylococcus aureus* was different.

Also, the minimum inhibitory concentrations of the methanol and ethanol extracts of steamed cola nut were observed between, 60 - 30mg/ml (*Pseudomonas aeruginosa*) and 60 – 30mg/ml (*Staphylococcus aureus*), and 60 - 30mg/ml (*Pseudomonas aeruginosa*) and 100 – 80mg/ml (*Staphylococcus aureus*) respectively. These observations show that methanol and ethanol had similar MIC with respect to *Pseudomonas aeruginosa*, each between 60 - 30mg/ml. The methanol extract showed a better inhibitory effect on *Staphylococcus aureus* at concentrations between 60 - 30mg/ml while the ethanol extract an inhibitory effect at concentrations between 100 – 80mg/ml.

The minimum inhibitory concentrations of the methanol extract of roasted cola nut that inhibited the growth of the microbes was observed between the concentrations 60 – 30mg/ml (*Pseudomonas aeruginosa*) and 60 – 30mg/ml (*Staphylococcus aureus*) whiles that of the ethanol extract was between the concentrations 60 – 30mg/ml (*Pseudomonas aeruginosa*) and 120 - 100mg/ml (*Staphylococcus aureus*). These observations show that the methanol and ethanol extracts show similar inhibitory effects at lower concentrations on *Pseudomonas aeruginosa*, each with MIC of 60 – 30mg/ml. The inhibition on *Staphylococcus aureus* was however different. The methanol extract had a better inhibitory effect on Staphylococcus aureus at 60 - 30mg/ml while the ethanol extract showed an inhibitory effect at 120 – 100mg/ml. The differences in the MIC's of the extracts could be attributed to the variation in the concentrations of the various phytochemicals in the extracts.

Comparing the minimum inhibitory concentration of the methanol and ethanol extracts of the raw, steamed and roasted cola nut, it was observed that both the ethanol and methanol extract of raw cola nut had a better MIC on *Pseudomonas aeruginosa* (30 - 15) compared to the methanol and ethanol extracts of steamed and roasted cola nut on *Pseudomonas aeruginosa*, 60 – 30mg/ml (*Pseudomonas aeruginosa*) and 60 - 30mg/ml (*Pseudomonas aeruginosa*) respectively. For *Staphylococcus aureus,* the methanol and ethanol extracts of raw cola nut, the methanol extract of steamed cola nut and the methanol extract of roasted cola nut all had the same MIC (60 - 30mg/ml) which was better than the ethanol extract of steamed cola nut (100 -80mg/ml) and the ethanol extract of roasted cola nut (120 – 100mg/ml). The overall comparison showed that both methanol and ethanol extracts of raw cola nut exhibited better inhibitory effect at lower concentrations against *Pseudomonas aeruginosa* compared to that of steamed and roasted cola nut. For *Staphylococcus aureus,* the methanol and ethanol extracts of all the extracts

showed similar MIC (60 – 30mg/ml) except for the ethanol extracts of steamed and roasted cola nut which showed (100 - 80mg/ml) and (120 – 100mg/ml). Also, the MIC of the standard (amoxicillin) compared to the extracts showed an MIC of 10.50mg/ml on the two microbes, which was better than that of the extracts. The extracts of raw cola nuts therefore have promising potentials against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

6. Conclusion

The methanol and ethanol extracts of raw, steamed and roasted cola nut contained phytochemicals such as alkaloids, saponins, flavonoids, terpenoids, tannins and phenols. It has also been evaluated that both the methanol and ethanol extracts of raw, steamed and roasted cola nut have prospective antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, the methanol extracts of both plants were generally more effective than the ethanol extracts. The range of the mean zones of inhibition of the methanol extracts of raw, steamed and roasted cola nut at 200mg/ml on *Pseudomonas aeruginosa* and *Staphylococcus aureus* were 15.00 ± 0.00mm and 10.00 ± 0.00mm, 7.00 ± 0.00mm and 8.00 ± 00mm, and 6.00 ± 0.00mm and 7.00 ± 0.00mm respectively. That of the ethanol extracts of the three plant samples at 200mg/ml *Pseudomonas aeruginosa* and *Staphylococcus aureus* were 13.00 ± 1.00mm and 10.50 ± 1.50mm, 8.50 ± 0.50mm and 6.50 ± 1.50mm, 5.00 ± 1.00mm and 6.00 ± 2.00mm respectively. An increase in the concentration of each of the extracts corresponded with an increase in the mean zone of inhibition.

Also, results of the minimum inhibition concentrations showed that both the methanol and ethanol extracts of raw cola nut were generally effective against the two microbes at low concentrations compared to those of the steamed and roasted cola nut. The standard drug (amoxicillin) was generally more effective against the two microbes compared to the extracts of the raw, steamed and roasted cola nut.

The presence of phytochemicals such as alkaloids, flavonoids, saponins, tannins, terpenoids and phenols which are known to have antimicrobial properties could be responsible for the inhibitory activity of the extracts. Hence, these experimental components could be beneficial in the management or treatment of various microbial infections caused especially by *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Recommendations

Research is required on toxicity of cola nuts to enable applications of a future product.

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