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The Phytochemical Properties and Antimicrobial Potentials of Aqueous and Methanolic seed Extract of *Cola nitida* (Vent.) and *Cola acuminata* (Beauvoir) grown in South West, Nigeria

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Abstract: The present study was undertaken to investigate the phytochemical constituents of two Nigerian grown kolanut and their antimicrobial properties. Extract of the seed of *Cola nitida* and *Cola acuminata* were used to determine the phytochemical constituent of aqueous and methanol nut extract and their solubility in the two solvents. Aqueous and methanol used reveals the solubility of these exrtacts in different solvent and the presence of secondary metabolites in the kola nut. Qualitative screening of the extracts of *C. acuminata* and *C. nitida* for phytochemicals revealed the presence of alkaloid, saponins, tannins, flavonoids, glycoside, steroid, saponins glycoside, cardiac glycoside and the absence of anthraquinones in all the extracts. Quantitative analysis shows that the methanolic extracts *C. acuminata* and *C. nitida* have the highest percentage of Saponins**:** (>10%) than the aqueous extract of the preparations. The disc method was used to determine the inhibitory effect of kolanut on the test microorganisms. Sensitivity patterns of zones of inhibition exhibited by the crude methanol and aqueous extract of *Cola nitida and Cola acuminata* shows that the nut possess relative degree of inhibitory effects against the test microorganisms: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Lactobacillus* respectively.. The zones of inhibition for *K. pnemonia* was the highest with 4mg/ml methanol extract of C. nitida and least for *E.coli.* As for *C. acumunata,* zones of inhibition for Lactobacillus was the highest with 4mg/ml methanol extract and least for *P. aeruginosa*. Highest zones of inhibition progressively increase from methanol to aqueous extract as their diameter of inhibition increases with increase in their concentration. The overall implication of the antimicrobial potentials of *C.nitida* and *C . acuminata* in the development of drugs against infectious diseases and subsequent health improvement was highlighted.
Keywords: Phytochemical properties, antimicrobial activity, *Cola nitida*, *Cola acuminata*, drug development

INTRODUCTION

Phytochemicals are compounds that occur naturally in plants. They contribute to the organoleptic (color, flavor and smell) properties of plants. In addition, they form part of a plant's natural defense mechanism against diseases [1]. Their medicinal values of phytochemicals to human health and disease prevention have been reported by a number of investigators [2-5]. In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind [6]. The World Health Organization strongly advocates the use of traditional medicine provided they are proven to be efficacious and safe [7]. In developing countries, the greater proportion of the populace lives in abject poverty and some are suffering and dying for want of safe water and medicine without an alternative for primary health care [8]. There is therefore the need to look in wards to search for herbal medicinal plants with the aim of validating their ethno-medicinal use and therapeutic potentials.

Moreover, the production of free radicals in living organisms is a vital phenomenon for the cell regulated through various biochemical or enzymatic detoxification process [9]. The free radicals are reported to be involved in many serious illnesses (such as heart disease and cancer) and constitute even aggressive form factors to DNA, proteins and other macromolecules [10]. It was necessary therefore that some of the plants used as forlore remedies for treating infectious diseases be investigated in order to validate their efficacy. However, some of the medicinal plants have been over harvested and are threatened with extinction thereby bringing to the fore the need to conserve these useful plants [6]. This can only be done with sufficient information that supports their efficacy (usefulness). On the other hand, the usage of traditional medicine has

met criticism because it has been associated with spiritism and backwardness. Scientific information was necessary to change this school of thought, and encourage people to use herbal medicine which is cheaper and within the reach of the vast majority of the population who are poor. This is particularly so because the of the challenge of drug-resistant pathogens being faced by the use of synthetic and orthodox therapy.

Kola nut (*Cola* spp.) belongs to the S*teruliacea* plant family with over 20 species native to the Africa tropical rain forest [11-13]. *Cola nitida* and *Cola acuminata* are the most common *Cola* species used. These species are sources of caffeine in processing and pharmaceutical industries and often chewed by some ethnic's group settings as stimulants [14, 15]. The presence of other chemicals in kola nuts such as kolanin and theobromine also makes them suitable for use in drug preparation[15, 16] . In addition, research has shown some potential uses of kola nut in the production of wine, chocolate and many non-alcoholic beverages [17-19].

The research into the discovery of new potent drugs from plants is based on the use of plant bio principles from extracts and essential oils to treat infections or diseases[1]. Since prevention is a more effective approach than treatment for chronic diseases, constant supply of phytochemical containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defense mechanism as this can help in reducing the risk of chronic diseases in humans. This work is thus targeted at investigating the phytochemical constituents of two Nigerian grown kolanuts and its potential antimicrobial effects on selected pathogens.

EXPERIMENTAL

Samples Collection

Five kilograms (5kg) each of *C. nitida* and *C. acuminata* freshly harvested was purchased from Eleyewo-daily-Market in Akungba-Akoko Ondo State, Nigeria. The nuts were authenticated by the Curator of the Herbarium of Biological Sciences department, Ahmadu Bello University, Zaria where the voucher specimens were deposited for reference. The nuts was washed with tap water and air dried under the shade, and later kept in an ambient container. They were thereafter transported to the Research Laboratory of Biochemistry department, Kaduna State University (KASU) till commencement of the study.

Samples Preparation

The seeds were cut into pieces and allowed to dry at room temperature. The dried materials were grounded to fine powder using electric blender, and later sieve. The extract was kept in a sterile plastic bag at temperature of 25± 2^{o}C for further use.

Preparation of Extracts

One hundred (100g) of each *Cola nitida*, and *Cola acuminata* were separately placed in a round bottom flask containing one liter of methanol and distilled water respectively. This was followed by mixing and agitation for six hours and it was allowed to stand for 24hours. The mixtures was filtered using muslin cloth and concentrated into powder(solid) by subjecting to heat using hot plate; the powdered dry extracts was scrapped off using spatula. The dried extract obtained was used directly for qualitative phytochemical screening.

Chemicals/ Reagent

Meyer's reagent, Wagner's reagents, Hydrochloric acid, Sodium chloride, potassium ferrocyanide, Ferric chloride, sodium hydroxide Fehling solution, methanol, acetic anhydride, were products of BDH Chemicals Ltd, Poole, England. Sulphuric acid, barium chloride, diethyl ether , n-butanol was procured from Sigma Chemical Co. St Louis, USA All other reagents used were of analytical grade and supplied by a reputable chemical manufacturers in the purest form available.

Collection of clinical isolate

Pure isolates of *Esherichia coli*, *Staphylococcus aureus*, *Lactobacillus*, *Klebsiella pneumonia*, *Pseudomonas auraginosa* was collected and authenticated in Microbiology laboratory of Microbiology department, Kaduna State University Kaduna and the identity was reconfirm using appropriate biochemical test. For the experiments, the bacterial isolates were first subcultured in nutrient broth (Oxoid, Ltd) and incubated according to standard procedure.

Preparation of 0.5 McFarland standards:

Solution A was prepared by adding barium chloride ($BaCl_2$, $2H_2O$) to 100ml distilled water. Solution B was prepared by adding 1ml of sulphuric acid (H_2S0_4 (0.36N) to 100 ml of distilled water. Then 0.5 ml of solution A was added to 99.5 ml of solution B, mixed well and distributed in test tubes with a screw cap. The cap is closed tightly to avoid evaporation. The mixture was stored in the dark. The solution was agitated vigorously before using it. After standardization of bacterial suspension, a sterile cotton swab was immersed in it and was rotated several times with firm pressure on the inside wall of the tube to remove excess fluid.

Phytochemical Screening of Plant Extracts

Phytochemical Screening

Phytochemical screening of extract was based on methods described by Trease and Evans [20] with some modifications. In this method, phytochemical screening was conducted to qualitatively determine the

presence or absence of the following phytochemicals in *C. nitida* and *C. acuminata* respectively. These are: Alkaloids, Tannins, Flavonoids, Saponins, Anthraquinones, Glycosides, Steroids, Saponins glycosides, Cardiac glycosides and Volatile oils.

Alkaloids

Exactly 0.5g of each extracts were stirred with 10 ml of 10% hydrochloric acid and allowed to stand overnight and then divided in to two parts for the following test.

a. 2 drops of Meyer's reagent was added to 1ml of the extracts, appearance of a creamy precipitate was taken as an evidence of the presence of alkaloids.
b. 2 drops of Wagner's reagent was added to 1 ml of the extracts. A reddish brown precipitate observed in each test tube indicated the presence of alkaloids.

Tannins

In this method, few drops of $FeCl_3$ (%w/v) solution was added to 3ml of the extracts in a test tube followed by shaking. A dirty green or dark blue coloration confirmed the presence of tannins.

Flavonoids

In this method, one millilitre of the extracts was treated with 1 ml of dilute NaOH. The presence of a cloudy precipitate confirms the presence of flavonoids.

Saponins

This was determined according to the method described by El-Olemy *et al* [21] and Harbone [22] In this method:

a. Five (5ml) of distilled water was added to 2ml of the extracts in a test tube and shaken vigorously. The formation of foams or stable frothing following the shaking indicated the presence of saponins.
b. 0.5g of each extracts were separately shaken with distilled water in a test tube, followed by heating in a water bath to boiling point. Persistent frothing on warming confirms the presence of saponins.

Cardiac Glycosides

This was done on the basis of Keller-killiani's test, to one of extract, 2ml of 3.5% ferric chloride solution is added and allowed to stand for one minutes. 1ml of conc. H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface indicated the presence cardiac glycoside.

Anthraquinones

In this method, two milliliters of 10% hydrochloric acid were added to the extract in a test tube and boiled for about two minutes. Equal amount of chloroform was added to the test tube and vortexes twice, the chloroform layer was pipette out and then equal volume of 10% ammonia was added. A pinkish red colour observed in upper layer indicated the presence of anthraquinones.

Glycosides

In this method exactly 2.5ml of 50% sulphuric acid was added to 5ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes, cooled and neutralized with 10% NaOH Then 5ml of Fehlings solution was added and mixture was boiled. A brick-red precipitate was observed which indicate the presence of glycosides.

Saponin Glycosides

This was determined according to the method described by El-Oley *et al*.,[21] In this method, to 2.5ml of the extract was added 2.5ml of Fehling's solution A and B. A bluish green precipitate showed the presence of Saponin glycosides.

Volatile Oils

In this method one1ml of the fraction was mixed with dil. HCl. A white precipitate was formed which indicated the presence of volatile oils.

Steroids

In this method exactly 2ml of acetic anhydride were added to 0.5g water extract of each sample with the addition of 2ml H_2SO_4. A colour change from violet to blue or green indicated the presence of steroids.

Quantitative Phytochemical Analysis

Alkaloid Determination

This was determined according to the method described by Harbone [22] with some modifications. In this method, exactly 2.5g of the samples were weighed into a 250ml beaker and 100ml cef 20% acetic acid in ethanol/methanol/water was added and covered to stand for 4 hours. This was filtered and the extract was concentrated using a water bath to one quarter (1/4) of the original volume. Concentrated ammonium hydroxide was completed the whole solution, was allowed to settle and the precipitate was collected by filter. The residue is alkaloid, which was dried and weighed.

Tannin Determination

This was determined according to the method described by Harbone [22] with some modifications. In this method, exactly 250g of the samples was weighed into 100ml plastic bottle 25ml of ethanol/methanol/distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipette out into a tube and mixed with 3ml of Ferric chloride in 0.1m HCl and 0.08ml

potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelengths, within 10 minutes of colour development. A blank sample was prepared and the color also developed and was at the same wavelength. A standard was prepared using tannin acid to get 100ppm and measured.

Saponin Determination

This was determined according to the method described by El-Olemy *et al* [21] with some modifications. In this method, exactly 20g of each ground plant samples were dispersed into 200ml conical flask contain 20% aqueous ethanol/methanol/water. The suspension was heated over a hot water bath for 4hrs with continuous stirring at about 55^0c. The mixture was filtered and the residue was re-extracted with another 200ml of 20% ethanol/methanol/water. The combined extracts were reduced to 40ml over water bath at about 90^0c. The concentrated was transferred into a 250ml separated funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated by measured 60ml of n-butanol was added and combined n – butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride (NaCl). The remaining solution was heated in a water bath. After evaporation, the samples were dried in a oven to a constant weight, and saponin content was determined.

Flavonoid Determination

This was determined according to the method described by El-Olemy *et al.*,[21] with some modifications. In this method, exactly 25g of each plant samples were extracted repeatedly with 100ml of 80% aqueous methanol/ethanol/water at room temperature. The whole solution was filtered through Whitman filter paper No.42 (125mm). The filtrate was later transferred into crucible and evaporated by dryness over water bath and weighed.

Phenol content determination

This was determined according to the method described by Harbone [22] with some modifications. In this method, the fat free sample was boiled with 50mL of ether for the extraction of the phenolic component for fifteen min. five mL of the extract was pipette into 50mL flask and then 10mL of distilled water was added. Two milliliter of ammonium hydroxide solution and 5mL of amyl alcohol were also added to the sample and made up to the mark. It was left to react for 30min for colour development; the absorbance was measured at 550nm.

Pathogenic test

The isolates of *Esherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia, Pseudomonas auraginosa* were subjected to catalase and coagulase test using standard procedure.

Sensitivity testing of aqueous and methanolic extracts of C. nitida and C. acuminata on bacterial isolates

The agar well diffusion technique of Kirby-Bauer was employed to test the antimicrobial effects of *Cola acuminata and Cola nitida* extract as recommended by NCCLS [23, 24].

In this method, the Muller-Hinton agar was poured into sterilized Petri-dishes, allowed to solidify for 30 minutes. The test organisms was inoculated onto the sterile agar plates and spread by a sterile cotton swab so as to achieve a confluent growth. Four wells of 8.0 mm in diameter each was aseptically bored using a sterile cork borer on each agar plate. On each agar plate, about 0.3mls of the extract of *Cola nitida and Cola acuminata* of varying concentration was added to the wells, at various concentrations .The same procedure was repeated for the aqueous and methanol seed extract respectively. The plates were then be incubated at 37°C for 24hrs. Effect of the extract was assessed by measuring the diameters of zones of inhibition to the nearest millimeter, and then compared with the standard Kirby - Bauer chart.

DATA ANALYSIS

Data were analyzed using simple percentages, mean, standard deviation and student t-test at $P< 0.05$ using Statistical Package for Social Sciences (Version 13.0) soft ware (SPSS, Chicago, IL.)

RESULTS

Fig. 1 and Fig. 2 pix adapted from Dah-Nouvlessounon *et al* [35].

Table 1 shows the result for the qualitative analysis of the phytochemicals screened with methanol and water. In these screening process alkaloids, tannins, saponins, flavonoids, steroids and glycosides are present in varying amount in methanol and aueous extract respectively. Anthraquinones was completely absent in the species tested.

Table 2 shows the quantitative analyses of the phytochemicals present in the *Cola spp*. It shows the presence of phytochemicals such as alkaloid, saponin, tannin, flavonoid and phenol in both extract with the methanol seed extract exhibiting a high significant difference ($P<0.05$) than the aqueous extract,.

Table 3 shows the antimicrobial activities of methanolic and aqueous seed extract of *C. nitida* at concentrations of 4,3,2 and 1 mg/ml tested against the some selected bacteria. There was a greater zone of inhibition when tested against *Klebsiella pneumonia* (21 mm) at 4 mg/ml concentration, *while Lactobacillus* had the lowest with 16.5 mm which is not significantly higher ($P< 0.05$) than the aqueous extract.

Table 4 shows the antimicrobial activity of methanolic seed extract of *C. acuminata* at Concentrations of 4, 3,2 and 1 mg/ml tested against the same bacteria. The extract has greater inhibition zones against *lactobacillus* at 4mg/ml extract (22.5mm) while *P.aeruginosa* had the lowest with 18.5mm which is significantly higher($P< 0.05$) than the aqueous extract.

Fig-1: variety and cotyledon number of *C. nitida* used in the study

Fig-2: variety and cotyledon number of *C. acuminata* used in the study

Table 1: Qualitative Phytochemical analysis of *C. nitida* and *C. acuminata*

Phytochemical constituents	Aqueous extract		Methanolic extract	
	C. nitida	*C. acuminata*	*C. nitida*	*C. acuminata*
Alkaloid	+++	+++	+++	+++
Saponins	+++	+++	++	++
Tannins	+++	+++	+++	+++
Flavonoids	+	++	++	++
Glycoside	+++	+++	+++	+++
Steroid	+++	+++	+++	+++
Anthraquinones	-	-	-	-
Saponin glycosides	+++	+++	+++	+++
Cardiac glycoside	+	+	++	++
Volatile oil	+	+	++	++

Key: +++ = phytochemicals present in high concentration, ++ = phytochemicals moderately present, + = trace, - =, phytochemicals not detected; *C. nitida = (Cola nitida) and C. acuminata = (Cola acuminata).*

Table 2: Quantitative Phytochemical Content of *C. nitida and C. acuminata*

Sample	Solvent	Alkaloid	Saponin	Tannin	Flavonoid	Phenol
C. nitida	Aqueous	0.20±0.01	08.10±0.12	0.36±0.02	0.32±0.00	0.29±0.006
	Methanol	0.26 ±0.04	10.01±0.00	0.46±0.01	0.49±0.02	0.31±0.009
C. acuminata	Aqueous	0.20±0. 00	08.40±0.07	0.44±0.00	0.46±0.01	0.29±0.022
	Methanol	0.30±0.08	10.20±0.10	0.34±0.10	0.32±0.06	0.31±0.069

Results are mean ± standard deviation of triplicate determinations

Key: *C. acuminata = (Cola acuminata), and C. nitida = (Cola nitida)*

Table 3: Sensitivity patterns of Zones of Inhibition exhibited by the crude methanol and aqueous extract of *C. nitida* on some pathogens at different concentrations

Bacteria	Solvent	Zones of Inhibition (mm*)			
		4mg/ml	3mg/ml	2mg/ml	1mg/ml
Staphylococcus aureus	Methanol	18.0	16.5	13.5	12.0
	Aqueous	16.5	15.0	13.5	12.5
Esherichia coli	Methanol	16.5	12.5	11.5	10.5
	Aqueous	13.0	12.5	11.0	10.5
Pseudomonas aeruginosa	Methanol	18.0	16.5	12.5	12.0
	Aqueous	14.0	12.5	12.0	11.0
Klebsiella pneumonia	Methanol	21.0	19.0	17.0	13.5
	Aqueous	15.5	14.5	13.5	12.5
Lactobacillus	Methanol	16.5	13.5	12.5	10.5
	Aqueous	14.0	13.5	11.5	10.5

Key: mm = millimeters, mg/ml = milligram per millimeter, *C. nitida = Cola nitida*
***** Result significant at P < 0.05

Table 4: Sensitivity patterns of Zones of Inhibition exhibited by the crude methanol and aqueous extract of *C. acuminata* on some pathogens at different concentrations

Bacteria	Solvent	Zones of Inhibition (mm*)			
		4mg/ml	3mg/ml	2mg/ml	1mg/ml
Staphylococcus aureus	Methanol	19.5	18.5	13.0	12.5
	Aqueous	13.5	12.0	11.5	10.5
Esherichia coli	Methanol	21.5	19.5	15.5	13.5
	Aqueous	16.5	13.5	12.0	10.5
Pseudomonas aeruginosa	Methanol	18.0	15.0	14.5	13.0
	Aqueous	13.5	12.5	12.0	11.0
Klebsiella pneumonia	Methanol	19.0	17.5	14.0	10.5
	Aqueous	15.0	13.5	12.5	11.5
Lactobacillus	Methanol	22.5	19.5	13.0	12.5
	Aqueous	12.5	11.5	11.0	10.5

Key: mm = millimeters, mg/ml = milligram per millimeter, *C. acuminata = Cola acuminata*
* Result significant at P <0.05

DISCUSSION

The goal of the present study is to investigate the phytochemical properties of two Nigerian grown kolanuts in aqueous and methanol seed extracts and their antimicrobial effects on selected test microorganism. A substantial body of literature is replete with the fact that medicinal plants constitute important source of new chemical substances with potential therapeutic benefits [11, 18, 25]. They have been shown to contain many biologically active compounds with medicinal properties and largely employed in developing countries [26]. They also produce a variety of natural products or secondary metabolites which are toxic in nature and are used as defence mechanism against microbial pathogens, insects, herbivores and other predators[27, 28]. In addition to the production of intrinsic antimicrobial compounds, it has been hypothesized that plants also produce multi-drug resistance inhibitors which enhance the activity of antimicrobial compounds [29]. These active constituents react with other bacterial pathogens and inhibit their growth [30]. Compounds isolated from natural origin such as medicinal plants are believed to have less side effects, tolerated by patients and can be afforded by most people since they are sold at a cheaper, reasonable price [31]. In the present study, qualitative phytochemical screening showed that that ethanolic extracts of *Cola spp* contain most of the phytochemicals like alkaloids,tannins, saponins, quinine and anthraquinone. A number of investigators are unanimous in observing that the medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body [3, 6, 25]. Phytochemical tests showed that aqueous and methanol extract of *C. nitida and C. acuminata* contained alkaloids, phenols, tannins, flavonoids, glycosides and saponins which could be regarded as the active principles that confer antimicrobial properties to this plant. Possession of alkaloids by some plants for instance has been shown to give distinct fungicidal activities and most likely to give the plants specific advantage against fungal attacks in the humid rain forest where such plants exist [32]. Also, Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties [1, 25] and bitterness [33]. The moderate amounts of saponin in

Cola spp investigated suggests that they may not be deleterious to the user as kolanut has long been known as a social fruit eaten by humans in all ages [19, 34]. This question of toxicity is however open to further testing. Several phenolic compounds like tannins, flavonoids present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant organisms. In addition, many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens [43].It is the presence of these compounds detected that probably account for the antibacterial activity of *C.nitida* and *C. acuminata* respectively. The relatively large amounts of saponins in *Cola spp* extracts investigated infer that the plants have biological functions such as protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and even tumor [2]. In a survey conducted in Benin republic, Dah-Nouvlessounon *et al* [35] found among others that *C.nitida* and *C. acuminata* respectively exhibit therapeutic effect on blood diseases, obstetrical diseases and digestive system disorders.

Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis [2].

The Kola extracts studied presented positive antimicrobial activity for *Staphylococcus aureus*, *Escherichia coli*,*Pseudomonas aeruginosa , Klebsiella pneumonia and Lactobacillus* The methanolic extracts of *Cola spp* are very potent in terms of activity because of the relative presence of bioactive components .Antimicrobial resistance is a major problem which the world is facing and is resulting in increased death rate. The multiple antimicrobial resistant bacteria cause severe problems that result in the complication of treatment of bacterial infections and this has been recognized by the World Health Organization [7]. Antibiotics are used as chemotherapeutic agents as they were believed to lead in the complete eradication of infectious diseases [36]. Despite the progress made in introducing new antibiotics, emergence of drug resistant strains cause failure of infectious disease treatment [37, 38]. Studies have found that antibiotic resistance occur as a result of an intrinsic mechanism that prevents bacteria from destruction[39, 40]. These bacteria usually do not have the structural cellular mechanisms that are needed in order for the antibiotic to act upon [41]. New effective strategy is therefore necessary for the management of resistance bacteria: one of which includes medicinal plants which may cater for about 80% of the vast populace that rely mostly on herbs for their medicines [42].

In our present study, the methanolic extract of the seeds demonstrated good activity against all the organisms with the highest zone diameter of inhibition of 22.5 mm against *lactobacillus.* The results for susceptibility testing of the extracts which are indicated in Tables 3 and 4 confirms the results of previous studies, which reported that methanol is an efficient solvent for extracting phytochemicals from *Cola spp* [43, 44]. The aqueous extract demonstrated poor activity relative to the methanol extract as were seen on the agar plate; which is in line with previous findings [45, 46]. This is an indication that water was not a good solvent.This could probably be that compounds responsible for bioactivity were not soluble in distilled water. Although methanol extract showed activity against both Gram - positive and Gram - negative, the Gram - positives were more susceptible with greater inhibition zones; these findings agree with previously published reports kolanuts from other climes [47, 48].

The activity of the methanol extract against both gram-negative and gram-positive organisms was of great interest because a more profound activity of several extracts is usually expected against gram-positive bacteria. This has been explained by the difference in cell wall composition, with gram-negative being complex [44, 49], The antimicrobial activity of *Cola spp* seeds extracts from South West Nigeria studied, showed a broad spectrum activity and this is in harmony with previous findings [3, 50]. This findings from present study is particularly instructive in pharmaceutics as extracts of *Cola spp* could be explored in the design of drugs to cure and also present the ravaging threats of these multi-drug resistant microgroorganism.

CONCLUSION

Our present study has demonstrated the presence of phytochemicals in *Cola spp* and these phytochemicals exhibit antimicrobial activity at all concentration in all the preparations. The findings no doubt indicates a promising potential of implicating the extracts of *Cola spp* in the design of drugs for treating infectious diseases. At present most pharmaceutical industries that produces drug synthetically are battling with the menace of drug resistance occasioned by these pathogens[13, 15, 17]. It is against this background that these industries now rely on the use of active ingredients from plants in the manufacture of their products. This development in our view is indeed encouraging as the cost of drugs to treat ailments occasioned by pathogens will be very much affordable and the attendant side effects minimized considerably. Apart from fuelling the literature on the bactericidal potential of *Cola species* grown in South West, Nigeria, our findings certainly suggest avenue for further investigation that will focus on the spectroscopic evaluation and identification of the non-volatile ingredients in *Cola spp* as well as the determination of

its toxicity through *in vitro* and *in vivo* studies with a view to ascertaining its safety parameters. This in fact is a prelude to subsequent initiation of clinical trials in the development of accessible and affordable efficacious therapy against infectious diseases.

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