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Antimicrobial Properties of Cola Nitida

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ABSTRACT: The antimicrobial evaluation of ethanol, methanol and aqueous extracts of *Cola nitida* on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was experimented. The assay was carried out by agar disc diffusion method. Results showed that the ethanol, methanol and aqueous extracts of the seed were found to be of a relatively higher effect having a zone of inhibition of 5mm against the samples of *S. aureus* than the other bacteria at the concentration of 400mg/mm. None of the extract was active against *P. aeruginosa*. *E. coli* showed a zone of inhibition of 3mm with ethanolic extract but showed a lower zone of inhibition with both methanol andaqueous extract. When compared to standard antibiotics such as septrin, amoxicillin, tetracycline, ampiclox and doxycycline, all the extracts had a high zone of inhibition of 5mm on *S. aureus* except for doxycycline which showed a higher zone of inhibition on all the test organisms. This antimicrobial property showed by the seed extracts on some of the organisms is an evidence of the ethno-medicinal uses of the plants due to the presence ofsome phytochemicals such as saponins, tanins, and alkaloids among others. For effective utilization of these Colaseeds as well as other relevant parts of the Cola plant, further research has been recommended.

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KEYWORDS: *Cola nitida*, agar disc diffusion extract, antibiotics, antimicrobial, phytochemicals

INTRODUCTION

Cola nitida commonly called Kola nut, ‘’Goru’’ or “Ajauru” in Hausa, ‘’Obi gbanja’’ or “Apo” in Yoruba, ‘’Oji’’in Igbo, “Ibon” in Efik, “Chigban bi” in Nupe and “Evbe” in Benin (Keay, 1989), is a member of the family Sterculiaceae. It is the product of a tree plant found in Sierra Leone, North Ashanti and Tropical West Africa including Nigeria, West Indies, Brazil and Java which grows about 40 feet high. Its fruits are usually produced from September to January as well as from June to July.

Cola species are often eaten by people and they are of high significance socially and traditionally. They are generally collected before maturity and left for several days in a file or immersed in water. Next, the pulpy tegument, which has disintegrated is eliminated (Bruneton, 1998).

The seeds incorrectly called kola nut comprise two cotyledons, is odourless and varies in colour between white and red (Evans, 2002). Microscopically, the outer layer consists a thin-walled polyhedral, parenchymatous cells 40-50 micrometers wide containing simple ovoid or spherical granules.

Fresh cola seeds which resemble conkers consist of large percent of water where observation according to Eijnatten in 1996 indicates that 60-70 percent of its weight is water. It contains about 2 percent caffeine and it ischewed by many people as stimulant. More also, it is used in the manufacture of dye, cola group beverages like coca-cola or pepsi.

It is highly valued for its perceived medical values which make it a highly desired product and as such *Cola nitida*as well as other plant products have been used since antiquity for medicinal purposes by diverse people and cultures throughout the world.

The phytochemical composition of *Cola nitida* seed is of obvious interest since it is reported to cure so many ailments. The key components are caffeine, thrombomine, tannins, phenolics, phlobaphene, anthocyanin, pigmentkola red, beteine, protein and starch. The medicinal uses of cola seeds are recognized officially indicated as toxic,stimulants, laxatives, sedative and diuretic (Eijnatten, 1996).

The essence of this study is to evaluate the antimicrobial potency of the extracts of *Cola nitida* using various solvents (ethanol, methanol and hot aqueous solvents) against some pathogenic bacteria that were selected basedon their prevalence in causing various infections in

man and other animals. They include *Staphylococcus aureus*,*Escherichia coli* and *Pseudomonas aeruginosa* which were subjected to sensitivity test at various concentrationsof each of the extracts.

MATERIALS AND METHODSMEDIA AND REAGENT

All the media used (MacConkey agar, Nutrient agar, Eosin methylene blue agar, Mannitol salt agar, Sensitivity agar) were prepared and sterilized according to manufacturer's instructions

SAMPLE COLLECTION AND PROCESSING

The seeds of *Cola nitida* were bought from market dealers in Benin City Nigeria and were botanically identifiedand confirmed by Mr. Akinnibonsun H. A. of the Department of Botany, Faculty of Life sciences, University of Benin, Benin City. The seeds were cut into smaller bits, air-dried for about 72 hours to reduce excess moisture and then ground into fine powder using the sterile mota.

EXTRACTION PROCEDURE

Aqueous extract

The aqueous, ethanol and methanol extracts were obtained by mixing the powdered seed materials of the plant (20g) with 100ml of the solvent in the ratio of 1:5 (weight/volume) at room temperature for 24 hours. The mixturewas filtered and the filtrate was evaporated to dryness and stored in a deep freezer until needed for the test (Fatope*et al*., 1993).

CULTURE AND IDENTIFICATION OF TEST ORGANISMS

Culture of isolate

The organisms used for the test were *Staphylococcus aureus, Escherichia coli, Pseudomonas auruginosa* (three different isolates for each test organism). All isolates that is each of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were inoculated on MacConkey agar, Eosin methylene blue agar and Mannitol salt agar; it was then incubated at 37°C aerobically for 24 hours after which it was examined for the presence of growth.

Colonies from the primary medium was further subculture on nutrient agar aerobically at 37°C then overnight thecultural characteristics on the colonies on each media were noted. Thereafter pure colonies were transferred to already prepared slants and stored for further use.

BIOCHEMICAL TEST

Catalase test, oxidase test, coagulase test, citrate utilization test, motility test as well as Gram stain were conductedon all isolates

PREPARATION OF INNOCULA AND IMPREGNATION OF PAPER DISC

The inoculum size of all bacterial isolate tested was standardized by the use of overnight broth cultures preparedby inoculating 3 loopfuls of well-isolated colonies of test bacteria in 10ml of peptone water and then incubated at37°C for 24 hours. Paper discs (10mm in diameter) were punched from No. 1 whatman filter paper using an officepuncher and impregnated with five different concentrations of the extract (400mg, 200mg, 100mg and 50mg and 25mg) after initially dissolving the stock extract with dimethylsulphuroxide (DMSO). Standard antibiotics were also available and were used as control in the course of the susceptibility test namely doxacyline, amplicox, amoxicilline, tetracycline and septrin.

BIOASSAY PROCEDURE

Agar Diffusion method as described by Kirby-Bauer (1966) and outlined by World Health Organization (WHO)in 1983 was employed. A peptone water culture of the test organisms was used to inoculate on a dry sterile sensitivity agar plate using a sterile swab and allowed to dry for about 15-30 minutes; the antibiotics discs as wellas the different concentration of each extract were placed on the agar using sterile forceps. Thereafter the plates were aerobically incubated at 37 °C for 24 hours. Diameters of the zones of inhibition were recorded in millimetersfor each concentration of the active fractions. '00' indicates a negative result and so no effect, diameter less than8mm (zone of inhibition) indicates low activity while that of 8mm and above indicates high activity.

DATA ANALYSIS

The data generated during this study were all subjected to various descriptive and inferential statistics and this statistical analysis was done using statistical package SPSS version 21 (Ogbeibu, 2005).

RESULTS AND DISCUSSION

Table 1 shows the morphological and biochemical characteristics of all the test organisms.
Table 2 gives the susceptibility pattern of the test organisms to ethanol soluble extract.
Table 3 gives the susceptibility pattern of the test organisms to methanol soluble extract.
Table 4 shows the susceptibility pattern of the test organisms to aqueous extract
And finally table 5 indicates the susceptibility pattern of these organisms to five different antibiotics ascontrol for the experiment.

Table 1: Results of morphological and biochemical test

	Cultural characteristic				Gram staining	Motility	Ca	Ox	Co	Ci
Organisms	**NA**	**MA**	**EMB**	**MSA**						
E. coli 1	1mm, flat, grey, entire	Deep pink colonies	Dark blue colonies with greenish metallic sheen	Nil	negative, short rod in chains	Motile	+	-	Nil	-
E. coli 2	1mm, flat, grey, entire	Deep pink colonies	Dark blue colonies with greenish metallic sheen	Nil	negative, shortrod in singly	Motile	+	-	Nil	-
E. coli 3	1mm, flat, grey, entire	Deep pink colonies	Dark blue colonies with greenish metallic sheen	Nil	negative, shortrod in singly	Motile	+	-	Nil	-
S. aureus 1	3mm, round, flat,cream	Nil	Nil	Yellow colonies	positive, cocci in clusters	Non- motile	+	-	+	-
S. aureus 2	3mm, round, flat, cream	Nil	Nil	Yellow colonies	positive, cocci in clusters	Non- motile	+	-	+	-
S. aureus 3	4mm, round, flat, cream	Nil	Nil	Yellow colonies	positive, cocci in clusters	Non- motile	+	-	+	
P. aeruginosa 1	2mm, round, flat, turns media green	Colourless grey, colonies the	Nil	Nil	negative, shortrod in pairs	Motile	+	+	Nil	+
P. aeruginosa 2	2mm, round, flat, turns media green	Colourless grey, colonies the	Nil	Nil	negative, shortrod in pairs	Motile	+	+	Nil	+

P. aeruginosa 3	2mm, flat, turns media green	round,Colourless grey,colonies the	Nil	Nil	negative, shortrod inPairs	Motile	+	+	Nil	+

Key: NA- nutrient agar, MA- macConkey agar, EMB- eosin-methylene blue agar, MSA- mannitol salt agar, Ca- catalase,Ox- oxidase, Co-coagulase, Ci- citrate. Numbers 1, 2, and 3 are used to represent isolates from different patients, nil- notconducted

Table 2: Antibacterial activity of ethanol soluble extract of *Cola nitida*

Test organism	Concentration of extract				
	400 (mg/ml)	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)
	Zone of inhibition (mm)				
Escherichia	00	00	00	00	00
coli 1					
Escherichia	00	00	00	00	00
coli 2					
Escherichia	03	02	02	01	01
coli 3					
Staphylococcusaureus 1	02	01	01	01	01
Staphylococcusaureus 2	05	04	03	03	01
Staphylococcusaureus 3	04	04	02	02	01
Pseudomonas aeruginosa 1	00	00	00	00	00
Pseudomonas aeruginosa 2	00	00	00	00	00
Pseudomonas aeruginosa 3	00	00	00	00	00
DMSO	00	00	00	00	00

Table 3: Antibacterial activity of methanol soluble extract of *Cola nitida*

Test organism	Concentration of extract				
	400 (mg/ml)	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)
	Zone of inhibition (mm)				
Escherichia	00	00	00	00	00
coli 1					
Escherichia	00	00	00	00	00
coli 2					
Escherichia	00	00	00	00	00
coli 3					

Staphylococcus aureus 1	01	00	00	00	00
Staphylococcus aureus 2	03	03	02	02	01
Staphylococcus aureus 3	05	03	03	02	02
Pseudomonas aeruginosa 1	00	00	00	00	00
Pseudomonas aeruginosa 2	00	00	00	00	00
Pseudomonas aeruginosa 3	00	00	00	00	00
DMSO	00	00	00	00	00

Table 4: Antibacterial activity of aqueous extract of Cola nitida

Test organism	Concentration of extract				
	400 (mg/ml)	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)
	Zone of inhibition (mm)				
Escherichia coli 1	00	00	00	00	00
Escherichia coli 2	00	00	00	00	00
Escherichia coli 3	00	00	00	00	00
Staphylococcusaureus 1	01	00	00	00	00
Staphylococcusaureus 2	03	03	02	02	01
Staphylococcusaureus 3	05	03	03	02	02
Pseudomonas aeruginosa 1	00	00	00	00	00
Pseudomonas aeruginosa 2	00	00	00	00	00
Pseudomonas aeruginosa 3	00	00	00	00	00
DMSO	00	00	00	00	00

Table 5: Antibacterial activity of selected antibiotics

Test organism	Concentration of antibiotics (400mg/ml) Doxycycline				
	Ampiclox	Amoxicillin	Tetracycline	Septrin	
	Zone of inhibition (mm)				
Escherichia	09	01	01	03	00
coli 1					
Escherichia	10	02	01	03	01
coli 2					
Escherichia	10	01	02	04	01
coli 3					
Staphylococcus	09	01	01	02	02
aureus 1					
Staphylococcus	10	01	01	03	01
aureus 2					
Staphylococcus	09	00	02	03	01
aureus 3					
Pseudomonas	11	01	01	04	01
aeruginosa 1					
Pseudomonas	10	01	02	04	02
aeruginosa 2					
Pseudomonas	10	01	01	03	01
aeruginosa 3					

The outcome of this study showed that the seed extracts of *Cola nitida* possessed antibacterial activity though in small amount when compared to the antibiotics used as control for the experiment. The antibacterial activity against the test organisms (*Pseudomonas auruginosa*, *Staphylococcus aureus* and *Escherichia coli*) varied significantly. This is due to the presence of some bioactive components presentin the extract such as alkaloids, tannins, saponins as well as caffeine, theobromine, theophylline, polyphenols, kolatine and other components as shown earlier in this study (Ebanu *et al*., 1999).

This study showed a moderately low susceptibility of *Staphylococcus aureus* to all the extracts producingthe widest zone of inhibition (5mm) at the highest concentration of 400mg/ml compared to other test organisms. This activity of the extracts on *S. aureus*; a Gram positive bacterium could be as a result ofthe interaction of the antimicrobial substances present in the extract with the molecular cell wall composition of the cell. This corresponds to the work reported by Sonibare *et al*., (2009) indicating that*Cola nitida* had a relatively low inhibitory effect on *Stapylococcus aureus* among other microorganisms.

On the other hand *Pseudomonas aeruginosa*, a Gram negative bacterium was highly resistant where it showed no effect in almost all instances of the extracts, this as well could be as a result of the plasmid- encoded resistance factors (R-factors) making them far less sensitive to the antimicrobial agents presentin the extracts Bryan *et al*., (1973). Yet still, *Escherichia coli* showed a very low susceptibility to aqueousextract whereas it showed completely no effect in the case of methanol extract. Furthermore, while *E. coli* 3 showed a low activity of 3mm at the highest concentration *E. coli* 1 and *E. coli* 2 were completelyresistant to ethanol extract. This variability in results probably could be as a result of the endogenous resistance of *E. coli* in their ability to adapt and survive in the presence of antibacterial agents as well assome antibiotics. That notwithstanding, the relatively low activity of these extracts give an indication thatthe species of Cola under investigation is of importance in trying to combat infection. However, when compared to standards, the antibiotic doxycycline showed a very high zone of inhibition of 10mm on virtually all the test organisms, septrin showed averagely 3mm whereas ampiclox, tetracycline and amoxicillin showed gave about 1mm and in few other cases no effect on the test organisms. This low activity of the extracts compared to the antibiotics used does not mean the absence of bioactivecompounds according to Adegboye *et al*., (2008) but that they are so low in

number due to the extractionprocedures involved probably giving rise to insufficient amount of the antibacterial agents needed to inhibit the growth of the test organisms. It is therefore recommended that a more reliable extraction procedure be employed such that will recover more of the antimicrobial agents within the extracts.

CONCLUSION

Cola nitida as deduced from the experiment has a very promising use as an antibacterial agent and for treatment of infections caused by the test organisms especially *Staphylococcus aureus*. Moreover for theusage of this medicinal plant (*C. nitida*) to be fully maximized, it is recommended that the seed as well as all parts of the plant should be properly assayed in the laboratory and further research is necessary to optimize the effective use of this agent in clinical practices.

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