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Phytochemical and Antimicrobial Properties of Crude n-Hexane and Methanol Extracts of *Cola acuminata* Nuts

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Authors' contributions

Author ENK carried out the research/work, wrote the first draft of the manuscript. Author ADB designed the study, supervised the research, read and approved the final manuscript. Author GM provided the ATCC strains and together with author ADB read the final manuscript.

Original Research Article

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ABSTRACT

Aim: To determine the phytochemical and antimicrobial properties of crude n-hexane and methanol extracts of *Cola acuminata* nuts against standard and clinical strains of pathogenic bacteria and fungi species implicated in various infections.
Place and Duration of study: Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria, between February 2009 and September 2009.
Methodology: All the microorganisms were tested for their susceptibilities to the plant extracts by means of agar diffusion (Kirby-Bauer) method according to the National Committee for Clinical Laboratory Standard guidelines. Minimum Inhibitory Concentrations were determined using the broth dilution method.
Results: The methanol extract showed activity on all 16 isolates tested with zones of inhibition in the range of 10 to 19 mm for fungi and 13 to 20 mm for bacteria while that of n-hexane was completely inactive. The minimum inhibitory concentration (MIC) of the methanol extract was between 62.5 and 500 µg/ml with the fungi species having higher values. Phytochemical analysis of *Cola acuminata* nuts reveals the presence of saponins,

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tannins, alkaloids and sugar. Griseofulvin, tioconazole and nystatin resistant *Candida sp* were sensitive to the methanolic extracts of *C. acuminata.* The kinetics of bactericidal and fungicidal studies show that 90% of *Staphylococcus aureus* and 99% of *Candida albicans* were killed within 3 h of contact time at a concentration of 250 µg/ml of the crude methanol extract of *Cola acuminata*.

Conclusion: In comparison with these antibiotics, the test plant extracts fared better in their antifungal activity and are capable of being a good replacement as alternatives if processed.

Keywords: Cola acuminate; Candida; antimicrobial; minimum inhibitory concentration; kinetics studies; minimum bactericidal/fungicidal concentration.

1. INTRODUCTION

The genus *Cola*, a tropical African genus of the Family *Sterculiaceae,* comprises about one hundred and twenty five species. *Cola* species are evergreen, mostly small or moderately sized trees although a few grow to 25 m high. A number of species are widely cultivated in tropical countries especially in Africa where they are found growing near the sea coast. Cola nuts are used mainly for their stimulant and euphoriant qualities. They have effects similar to other xanthine-containing herbs like cocoa, tea, coffee and guarana. However, the effects are distinctively different, producing a stranger state of euphoria and well being. They have stimulant effects on the central nervous system and heart. Animal experiments indicate that Cola nuts have analeptic and lipolytic (fat burning) properties and stimulate the secretion of gastric juices while human studies show Cola nuts have positive chronotropic and weak diuretic effects. In humans it enhances alertness and physical energy, elevates mood, increases tactile sensitivity, suppresses the appetite and is used in Africa as an aphrodisiac. Traditionally, the leaves, twigs, flowers, fruits follicles and the bark of *C. acuminata* are used to prepare a tonic as a remedy for dysentery, cough, diarrhea, vomiting [1] and chest pain [2]. The nuts have considerable potential for the development of new pharmaceuticals and foods [3]. The powdered nuts help to stop diarrhea and are used in French Equatorial Africa, externally for itch and ulcer [4]. There has not been any report on the antimicrobial spectrum of *C. acuminata* nuts. Hence, the objective of this research is to investigate the antimicrobial spectrum of *C. acuminata* using wide range of pathogenic isolates and its effectiveness in treating microbial infections.

2. MATERIAL AND METHODS

2.1 Plant Material and Preparation of Extracts

Cola acuminata nuts used in this research were collected from local traders at Oje market, Ibadan, Oyo state. The samples were identified and authenticated at the herbarium in the Department of Botany and Microbiology University of Ibadan as well as Forest Research Institute of Nigeria, Ibadan as *Cola acuminata* (F.H.I.108304).

Microorganisms used were standard (ATCC) strains of *Candida albicans* ATCC 90029, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6825, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* AS 1199, *Staphylococcus aureus* ASXY 212, *Enterobacter cloacae* ATCC 49141, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922*, Klebsiella pneumoniae* ATCC 35657, *Pseudomonas aeruginosa* ATCC 27853

and *Acinetobacter baumannii* ATCC 747 obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan and the clinical strains of *Candida albicans*, *Candida tropicalis*, *Candida krusei, and Candida glabrata* obtained from University College Hospital (UCH) Department of Microbiology, University of Ibadan, confirmed, subcultured in Mueller Hinton broth and agar.

2.1.1 Extraction

Powdered materials of the Cola nuts weighing 1000 g were soxhlet extracted with 1 L of n-hexane and methanol in succession for 6 h each. The different extracts were each concentrated until dry (n-hexane extracted yielded 12.8 g while methanol extract yielded 53 g) and the different residues of the *Cola* extracted were re suspended in hexane and methanol to a concentration of 2000 µg/ml and stored in air tight bottles at 4^{o}C in the refrigerator for use [5].

2.1.2 The Phytochemical screening

The samples were screened for the presence of secondary metabolites such as saponins, alkaloids, cardenolides, tannins, anthraquinone glycosides and reducing sugar [6].

2.2 Antimicrobial Susceptibility Test

All the test microorganisms were tested for their susceptibilities to the plant extracts by means of agar diffusion (Kirby-Bauer) method [7]. A 2000 µg/ml concentration of the *Cola acuminata* extract using n-hexane and methanol diluents was prepared and used for this test. A 1:100 over night broth culture of the fungal species in Trypton soy broth and Muller Hinton broth for bacteria were made by adding 0.1 ml of the broth into 9.9 ml of sterile distilled water from this mixture respectively. This is to standardize the inoculum to obtain cell dilution of 10^{-2}. 20 ml of sabouraud dextrose agar (SDA) and Mueller Hinton agar respectively in universal bottles were poured aseptically into separate sterile petri dishes and allowed to set. Using sterile Pasteur pipette, 1ml. of the 10^{-2} dilution of fungi *sp* were placed on the surface of each plate. These were spread uniformly with the aid of a sterile glass spreader. Each plate was tilted, and the excess suspension allowed to drain to an edge where it was removed by suction using a sterile Pasteur pipette. Wells of uniform diameter of 7 mm were punched in each plate by means of a sterilized cork borer. Sterile Pasteur pipettes were used to fill the wells with the 2000 µg/ml concentration of the test extract. The plates were left for an hour to allow for sufficient diffusion of the extract into the media before incubation. All plates were incubated at 25ºC for fungi and 37ºC for bacteria for 24 to 48 h. The diameter zones of growth inhibition were measured after the period of incubation with a meter rule. All plates were duplicated. The solvents alone were introduced to separate wells as control.

2.2.1 Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MICs) of the active extracts were determined by agar dilution method as described by Adeniyi et al. [8]. A 2000 µg/ml concentration of the extract was made as in the susceptibility test above. Double-fold serial dilutions of the extract were made by taking 2.5 ml from the stock solution into another bottle containing 2.5 ml of the solvent (methanol) and so on producing concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml respectively. Exactly 2 ml volume of the extract was added into 18 ml of sterile media maintained at 45ºC.They were properly mixed for even

distribution of the extracts within the agar and allowed to set. A loopful of the diluted susceptible overnight broth cultures were used to inoculate each of the solidified agar-extract mixture in duplicates. The plates were then examined for the presence of colonies after the incubation period of 24 to 48 h at 37ºC and 25ºC respectively. The least concentration that gave no visible colonies was taken as the minimum inhibitory concentration of the extract for the particular dilution of the organism.

2.2.2 Determination of the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Minimum fungicidal concentration (MFC) of the plant extract was determined. 0.5 ml extract at different concentration used in the MIC assay was added 0.5 ml of test organism in tubes. These were incubated at 25ºC for 48 h. Samples were streaked out from the tubes on to sabouraud dextrose agar to determine the minimum concentration of the extract required to kill the organisms. These concentrations were indicated by failure of the organism to grow on transfer to these media plates. The lowest concentration that prevented fungal growth after 48 h of incubation was recorded as the minimum fungicidal concentration (MFC). All tests were performed in duplicates to ensure accuracy. Agar plates without extracts and another agar plates without any inoculated organism were also incubated serving as positive and negative control plates respectively.

Bactericidal/Fungicidal activities of the extracts with time (kinetics) were determined with the viable counting technique employed for this purpose. From the MBC setup, 0.1 ml of the broth was taken and inoculated unto agar plates every 1 h interval and incubated. Presence or absence of growth was noted and colonies counted.

3. RESULTS AND DISCUSSION

Antibacterial and antifungal properties of the n-hexane and methanol extracts of *Cola acuminata* nuts were determined against four different *Candida* species both clinical and standard strains and nine standard strains of bacteria (Table 1). The methanol extract of *C. acuminata* was active against all the 16 isolates tested while the n-hexane extract was not active against any isolate. Also the solvents used as control showed no antimicrobial activity.

Table 2 shows the minimum inhibitory concentrations (MICs) and the minimum bactericidal/fungicidal concentrations (MBC/MFC) of the active methanol extract of *C. acuminata* against the test isolates. The values obtained showed that the MIC ranged between 25 µg/ml to 250 µg/ml for the bacterial isolates and 62.5 µg/ml to 1000 µg/ml for the fungal isolates. This indicates that the bacterial isolates were more susceptible to the cola extracts than the fungal isolates.

The antibiogram (Tables 3-4) showed that all the isolates were multi-resistant to various antibiotics (for bacteria isolates) and antifungal drugs (for fungal isolates).

The phytochemistry of the cola nuts revealed the presence of saponins, alkaloids, tannins, glycosides and reducing sugar (Table 5).

The bactericidal kinetics of the extracts against some selected organisms is shown in Figs. 1 and 2. The graph shows the percentage number of survivors decreasing with time.

Table 1. The Antimicrobial Activities of Crude Methanol and n-Hexane Extracts of *Cola acuminata* Showing Zones of Inhibition (mm), at a Concentration of 2000 µg/ml.

Test Organism	Zones of Inhibition of plant Extracts
	Methanol Extract (mm)
S. aureus ATCC 29213	15±0.50
S. aureus AS1199	25±1.00
S. aureus ASXY 212	17±0.00
P. aeruginosa ATCC 27853	19±0.50
E. cloacae ATCC 49141	16±0.00
E. faecalis ATCC 29212	18±0.00
E. coli ATCC 25922	13±0.00
C. parapsilosis ATCC 22019	15±0.00
C. albicans ATCC 90029	15±0.00
C. krusei ATCC 6825	17±0.50
K. peumoniae ATCC 35657	15±0.00
A. baumannii ATCC 747	13±0.00
C. glabrata (S1)	18±0.00
C. glabrata (S2)	19±0.50
C. albicans (S1)	17±0.00
C. albicans (S2)	15±0.00
C. krusei (S1)	16±0.50
C. krusei (S2)	15±1.00
C. tropicalis (S1)	10±0.00
C. tropicalis (S2)	15±0.00

Values are mean; ± standard error of means; (S1), (S2) = Clinical Strains
***N/B**: No inhibition was seen with n-hexane extracts of all the test organisms*
Diameter of Well = 7 mm

Table 2. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MIC/MFC) of Crude Methanol Extract of *Cola acuminate*

Test Organism	MIC (µg/ml)	MBC/MFC (µg/ml)
S. aureus ATCC 29213	125	250
S. aureus AS1199	250	500
S. aureus ASXY 212	250	500
P. aeruginosa ATCC 27853	62.5	125
E. cloacae ATCC 49141	62.5	125
E. faecalis ATCC 29212	16.23	125
E. coli ATCC 25922	31.25	62.5
A. baumannii ATCC 747	125	125
K. peumoniae ATCC 35657	125	250
C. krusei ATCC 6825	62.5	125
C. albicans ATCC 90029	250	250
C. parapsilosis ATCC 22019	250	250
C. glabrata (S1)	500	1000
C. glabrata (S2)	125	500
C. albicans (S1)	250	1000
C. albicans (S2)	250	250
C. krusei (S1)	-	-
C. krusei (S2)	500	1000
C. tropicalis (S1)	1000	1000
C.tropicalis (S2)	500	1000

(S1), (S2) = Clinical Strains; - = not determined

Table 3. The Sensitivity of Candida Species to Different Antifungal Drugs

DRUG	*C. albicans*			*C. parapsilosis*			*C. krusei*			*C. tropicalis*	
	S*	C1	C2	S*	C1	C2	S*	C1	C2	C1	C2
Griseofulvin 10 µg/ml	R	R	R	R	R	R	R	R	R	R	R
Tioconazole 10 µg/ml	R	R	R	R	R	R	R	R	R	R	R
Ketoconazole 4 mg/ml	S	S	S	S	S	S	S	R	R	R	R
Nyastatin 100,000 UI	R	R	R	R	R	R	R	R	R	R	R

Note: R = Resistance; S = Sensitive; S= Standard strains; C1, C2 = Clinical strains*

From this study, it was observed that the assayed *Cola* nut samples demonstrated antimicrobial activities against the tested organisms. This was shown in the varying zones of inhibition of the individual extracts on the pathogens *In-vitro*. The zones of inhibitions produced by the test plant extracts varied from one organism to another. The observed antimicrobial activities of the various crude extracts against the organisms may be as a result of the presence of secondary metabolites which are known to posses' antimicrobial activities [9]. It may also have involved complex mechanisms like the inhibition of the synthesis of cell wall, cell membrane, nucleic acid and proteins as well as the inhibition of the metabolism of nucleic acids [10]. The methanol extract of the cola species demonstrated higher antimicrobial activity against the test organisms while the n-hexane extracts had no antimicrobial activity. This could be because methanol is reported to be a better solvent for the consistent extraction of antimicrobial substances from medicinal plants when compared to other solvents such as ethanol, chloroform, n-hexane and aqueous solvent [11]. This finding agrees with the report of Shama et al. [13] who reported the antimicrobial activity of the masticatory *Cola acuminata.*

The findings on *C. acuminata* is of importance/significance, though chewed by many for many reasons especially their stimulating effect, they could be used in the treatment of oral candidiasis and other mycotic infections and bacterial diseases such as respiratory tract infections, urinary tract infections, wound sepsis, oral and dental infections since it possesses antimicrobial properties.

The observed sensitivity pattern of the test organisms is worthy of note. These organisms are widely known for their intrinsic resistance to the azole drugs and other antifungal drugs such as Amphotericin B and Griseofulvin hence, use of stronger antifungal agents in treatment is recommended. This explains the observation that the drugs employed in this research as controls (griseofulvin, tioconazole and nystatin), and to some extent ketoconazole, were observed to be inactive against the organisms tested. That is to say the organisms were resistant to these drugs. In comparison with these antibiotics, the test plant extracts fared better in their antifungal activities. This is an indication that the nut of this plant possesses a great potential as a substitute for these well-known antimicrobial agents.

Table 4. Susceptibility of the standard strain microorganisms to various antibiotics

Test Organism	Diameter zone of inhibition (mm) Gram Negative Disc						
	AMX 25 µg	COT 25 µg	NIT 300 µg	GEN 10 µg	NAL 30 µg	OFL 30 µg	TET 30 µg
S. aureus ATCC 29213	0±0.00	10±1.00	21±2.50	15±0.00	15±0.50	17±0.50	0±0.00
P. aeruginosa ATCC 27853	0±0.00	11±0.50	19±1.00	0±0.00	0±0.00	30±0.50	10±0.5
E. cloacae ATCC 49141	0±0.00	17±0.50	16±0.00	17±1.50	16±0.50	30±0.00	10±2.50
E. faecalis ATCC 29212	0±0.00	15±1.50	24±0.50	10±1.00	0±0.00	20±0.00	0±0.00
E. coli ATCC 25922	0±0.00	16±1.00	15±0.50	15±2.00	20±3.50	30±1.20	10±1.50
C. parapsilosis ATCC 22019	0±0.00	0±0.00	20±2.00	10±1.50	0±0.00	20±2.60	0±0.00
C. albicans ATCC 90029	0±0.00	14±1.50	20±2.00	18±2.5	0±0.00	26±0.50	15±0.00
C. krusei ATCC 6825	0±0.00	20±1.50	21±2.50	15±1.00	13±0.5	30±2.50	10±2.00
K. pueumoniae ATCC 35657	0±0.00	20±1.50	18±0.00	18±0.50	20±2.50	25±0.00	10±0.00
A. baumannii ATCC 747	0±0.00	20±1.50	18±0.50	8±1.70	15±0.00	28±1.00	6±0.50
S. aureus SA1199	0±0.00	18±1.00	18±0.00	17±1.50	0±0.00	30±3.50	10±0.00
S. aureus ASXY 212	0±0.00	0±0.00	16±1.00	0±0.00	0±0.00	0±0.00	0±0.00

Test Organism	Diameter zone of inhibition (mm) Gram Positive Disc							
	AUG 30 µg	AMX 25 µg	ERY 5 µg	TET 10 µg	CXC 5 µg	GEN 10 µg	CHL 30 µg	COT 25 µg
S. aureus ATCC 29212	0±0.00	0±0.00	0±0.00	17±0.00	0±0.00	16±0.00	22±2.00	26±3.00
P. aeruginosa ATCC 27853	0±0.00	0±0.00	0±0.00	15±0.50	0±0.00	19±1.50	25±2.50	20±1.00
E. cloacae ATCC 49141	15±1.50	0±0.00	0±0.00	18±0.50	0±0.00	20±0.50	26±2.50	25±0.50
E. faecalis ATCC 29212	18±0.50	20±0.5	14±0.50	20±0.00	12±0.50	24±0.50	18±0.00	23±2.50
E. coli ATCC 25922	10±0.00	10±1.5	0±0.00	20±0.50	0±0.00	21±1.50	24±3.50	0±0.00
C. parapsilosis ATCC 22019	16±1.50	25±0.5	0±0.00	0±0.00	0±0.00	20±0.00	12±0.00	0±0.00
C. albicans ATCC 90029	0±0.00	0±0.00	0±0.00	8±0.50	0±0.00	20±0.00	0±0.00	0±0.00
C. krusei ATCC 6825	15±2.50	18±0.5	0±0.00	0±0.00	0±0.00	18±0.50	14±0.00	0±0.00
K. pueumoniae ATCC35657	12±1.50	0±0.00	0±0.00	18±0.00	0±0.00	23±2.00	25±2.50	26±0.00
A. baumannii ATCC 747	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	19±1.50	0±0.00	0±0.00
S. aureus AX 1199	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	16±0.00	0±0.00	0±0.00
S. aureus SAXY 212	20±2.20	22±3.70	27±2.00	15±0.50	0±0.00	18±0.50	24±3.80	22±0.50

Amx-Amoxicillin, Cot-Cotrimoxazole, Nit-Nitrofurantoin, Gen-Gentamicin, Nal-Nalidixic acid, Ofl-Ofloxacin, Tet-Tetracycline, Aug-Augmentin, Ery-Erythromycin, Cxc-Cinoxacin, Chl-Chloramphenicol.

Table 5. Phytochemical screening of *Cola acuminata* nut

Plant	Test			
Cola Acuminata	Tannins +	Saponins +	Alkaloids +	Glycosides +

+ = positive reaction

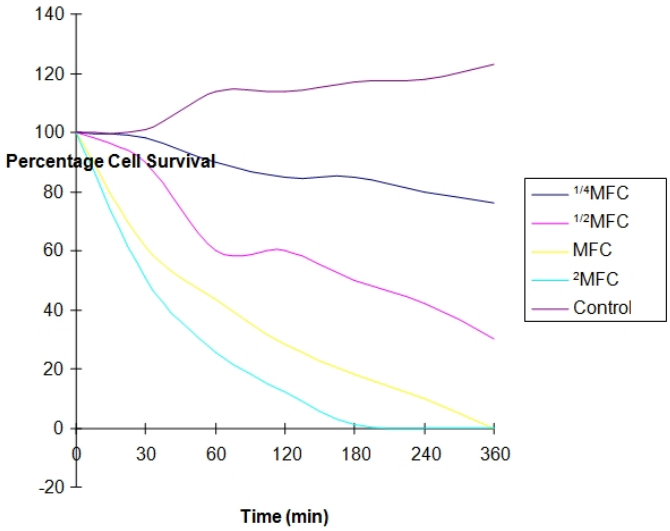

Fig. 1. Kinetic kill curve of *Cola acuminata* on *Candida krusei* ATCC 6825

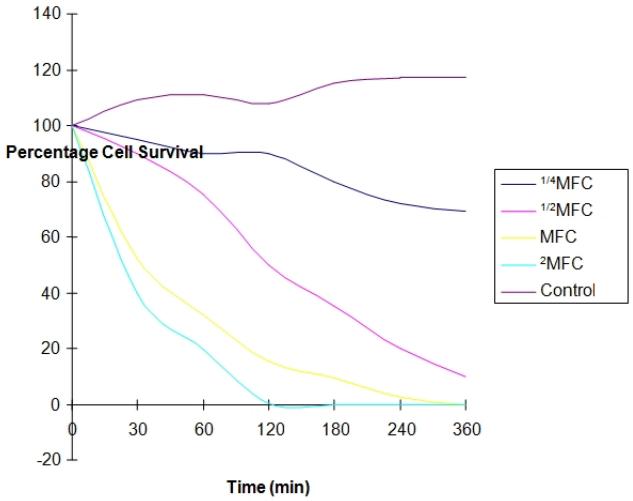

Fig. 2. Kinetic kill curve of *Cola acuminata* on *Staphylococcus aureus* ATCC 29213

4. CONCLUSION

It has not been reported that this plant employed for many uses in the African communities has any serious adverse toxic effects. Further research could be undertaken on it to formulate the active components into proper dosage forms.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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