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Full Length Research Paper

Potential Antimicrobial Activity of Cola acuminata Extracts against *Proteus mirabilis, Staphylococcus aureus,* and *Candida albicans*

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Ethanolic extract of the seed of *Cola acuminata* was evaluated for its antimicrobial and antifungal activities as compared to standard anti-microbial agents using *Proteus* mirabilis, *Staphylococcus aureus* and *Candida albicans* following standard microbiological procedures. Phytochemical screening of the seed extracts reveals abundance presence of Tannins, Cardiac glycosides, flavonoids and Phlobatanins and moderate presence of Alkaloids with a total absence of Saponins. At 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml concentrations of the extract used, *Staphylococcus aureus* and *Candida albicans* were susceptible to all except the later which was resistant to 25mg/ml concentration. *Proteus mirabilis* was resistant to all concentrations of the extract employed. *Staphylococcus aureus* was susceptible to vancomycin (30µg) yielding 18mm zone of inhibition as compared to the seed extract (20mm). *Proteus mirabilis* was susceptible to 10µg of Gentamycin (16mm), while Candida *albicans* was susceptible to Clotrimazole (50µg) yielding 17 mm zone of inhibition as compared to the seed extract (20mm). The Minimum inhibitory concentration of the extract was 50mg/ml and 25mg/ml for *Staphylococcus aureus* and *Candida albicans* respectively. This study shows that ethanolic extract of Cola acuminata is a superior antimicrobial agent against *Staphylococcus aureus* and *Candida albicans*, but not for *Proteus mirabilis* as compared to the drugs evaluated.

Keywords: *Cola acuminata,* antimicrobial, antifungal, *Proteus* mirabilis, *Staphylococcus aureus*, *Candida albicans.*

INTRODUCTION

Cola acuminata nut a capsule-shaped fruit belongs to the Sterculiaceae family that has its origin as West Africa and has been known to man for centuries. The nuts have been used by the African natives from immemorial as a necessity and a luxury. Before the emergence of western civilization and religion, cola nut played a very significant role in the daily life of the people of West Africa(Kiple and Ornelas, 2000; Iyere, 2011) including Nigeria. Nothing was considered complete without a cola nut. Cola was offered to a very important Guest as a mark of respect and recognition, agreements are never considered sealed without cola nuts shared, the gods cannot be appeased without cola nut. Similarly, marital rites are not complete without cola nut being presented (Iyere, 2011). Shrines are adorned with them as a means of sacrifice (Rätsch, 2005).

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It figured in compacts of friendship and a mark of hospitality as it was readily served to visitors, especially among the Igala People of Kogi State and Igbo tribe in eastern Nigeria as a sign of peace and acceptance of visitors (Iyere, 2011).

Some studies have revealed that the social significance of the use of the nut may lie in its being a very concentrated source of central nervous system (CNS) stimulation. In addition, the nut has been revealed to contain caffeine which may help in relieving migraine (Kiple and Ornelas, 2000), theobromine which act as a cerebral vasodilator and thought to relieve pain and neuralgia (Hirt and Mpia, 2001) and proanthocyanidin which is used as anti-trypanosome compound effective against *Trypanosoma bruci* (Kubata *et al*., 2005). Furthermore, traditional *Cola acuminata* nuts are also employed in the treatment of malaria and fever (Odugbemi, 2006), while its the leaves, flowers, fruits follicles and tree are used to prepare a tonic as a remedy for dysentery, cough, diarrhea, and vomiting and chest complaints (Irvine, 1961; Ayensu, 1978; Burkill, 1995).

Sundstrom, (1966) and Nickalls, (1986) in two very independent research revealed that the nuts of Cola acuminata also ease hunger and thirst, eliminate fatigue, provide energy by stimulating the muscles and nerves, in addition to enhancing intellectual activity. Presently, the dried nuts of Cola acuminata have been found useful in the production of some beverages and for pharmaceutical purposes (Ayodele, 1995).

Despite the extensive uses of the cola in folklore medicine and in the modern, traditional application very few studies have evaluated its antimicrobial potentials. This study is therefore aimed at evaluating the phytochemical components of *Cola acuminata* seeds and to determine the antimicrobial activities of its extracts against *Proteus species, Staphylococcus aureus, and Candida albicans.*

MATERIALS AND METHODS

Collection of Plant Materials.

The seeds of cola acuminata were purchased in Akpan Andem market, Uyo local Government Area, Akwa Ibom State, Nigeria, identified by a specialist in the Department of Pharmacognosy, Faculty of Pharmacy, the University of Uyo. It was peeled, cut into bits and then powdered using a sterile electric blender.

Test organisms

Standard isolates of *Staphylococcus aureus* (NCTC 10788), *Proteus mirabilis* (NCTC 8309) and *Candida albicans* (NCTC 3255) were supplied by the Microbiology Research division of Imperial Community Health Organization (I-CHO).

Antimicrobial disks

Paper disks of clotrimazole (50µg), gentamycin (10µg) and vancomycin(30µg) supplied by the Microbiology Research division of Imperial Community Health Organization(I-CHO).

Culture media

Sabouraud dextrose agar, Diagnostic sensitivity agar (DST) and nutrient agar, all Oxoid products were the media of choice employed in this study**.**

Extraction of Cola acuminata nut.

One hundred and twenty five grams (125g) of the *Cola acuminata* seed powder was extracted with 100ml of 60% ethanol over 72 hours. The mixture was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm).. The filtrate was put in a beaker and left to concentrate in a water bath (UNISCOPE, SM80IA) at 60 degrees Celsius.

Phytochemical Screening

The phytochemical analysis of the seed extract was performed in the department of Pharmacognosy, the University of Uyo using the methods described by Trease and Evans (1989), and Sofowora (1993).

Screening for Alkaloids

Saponin, tannins, phlobatannins, flavonoids and cardiac glycosides were all screened for following the methods described by Sofowora, (1993).

Determination of Antimicrobial Activity

The ability of the extracts to inhibit the growth of the clinical isolates was determined using Agar well diffusion method as described by Iroha *et al*., (2008).

Determination of Minimum Inhibitory Concentration of the Extract on Each of the Isolates.

The minimum inhibitory concentration (MIC) of the extract was determined using the tube dilution method described by (Cheesebrough, 2001). Different concentrations of the extract were prepared and standardized inoculums of the test organisms added. Control cultures without extracts were set up also and both control and experimental tubes were incubated at 37^0C for 18-24 hours. The MIC was reported as the lowest concentration extract that showed no visible growth.

Table 1. Phytochemical composition of the seeds of *Cola acuminate*

Components	Inference
Alkaloids	+
Saponins	-
Tannins	+++
Flavonoids	+++
Cardiac glycosides	+++
Phlobatannins	+

Key + = Present
++ = Present
+++ = Abundantly Present
- = Absent

Table 2. Antimicrobial Susceptibility of Standard Microorganisms to Ethanolic extracts of *Cola acuminata* and Antimicrobial agents showing zones of inhibition in millimeters (mm).

Extract	Susceptibility of Isolates (mm)		
Conc. (mg/ml)	***Staph aureus***	***Proteus* sp**	***Candida albican***
400	20	9	20
200	17	-	20
100	15	4	18
50	15	-	17
25	-	-	15
Antibiotic/antifungal			
Vancomycin (30µg)	18	NA	NA
Gentamycin (10µg)	15	16	NA
Clotrimazole (50µg)	NA	NA	17

Key: (-)= Negative
NA=Not applicable

RESULTS

Phytochemical Components of Cola acuminata.

The result of the phytochemical screening of *Cola acuminata* seed extracts is presented in Table 1. The plant seeds contained five phytochemicals. Alkaloids and Phlobatannins were present, while Tannins , Cardiac Glycosides and flavonoids were abundantly present. Saponins were absent.

Antimicrobial Susceptibility of Standard Microorganisms to Extracts of Cola acuminata Seeds.

The effect of the susceptibility of the standard microorganisms' to varying concentrations of 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml of the ethanolic extract of Cola *acuminata* and standard antimicrobial drugs is presented in Table 1.

At 400mg/ml concentration the seed extract inhibited the growth of *S. aureus* producing 20mm zone of inhibition, 200mg/ml concentration yielded an inhibition zone of 17mm. Similarly, 100mg/ml and 50mg/ml concentrations yielded the same zones of inhibition of 15mm. *S. aureus* was resistant to the 25mg/ml concentration of the ethanolic extract.

Proteus mirabilis yielded insignificant zones of inhibition at 400mg/ml (9mm) and 100mg/ml (4mm), concentrations, with no activity at all at 200mg/ml, 50mg/ml, and 25mg/ml concentrations. *Candida albicans* showed the highest zones of inhibition of 20mm, 18mm, 17mm and 15mm at the concentration of 400mg/ml, 200mg/ml, 50mg/ml and

Table 3. Minimum Inhibitory Concentration of Ethanolic extracts of *Cola acuminata* of Standard Microorganisms'.

Extract	Susceptibility of Isolates		
Conc. (mg/ml)	*Staph aureus*	*Proteus* sp	*Candida albicans*
400	+	_	+
200	+	_	+
100	+	_	+
50	+	_	+
25	_	_	+
12.5	_	_	_

Key (+)= Growth
(-)= No growth

25mg/ml respectively which shows that the seed extract of *C. acuminata* was susceptible to the organism.

Similarly, 30µg of vancomycin yielded an inhibition zone of 18mm against *Staphylococcus aureus*, while 10µg of gentamycin produced inhibition zones of 15mm and 16mm against *Staphylococcus aureus* and *Proteus mirabilis* respectively. Fifty micrograms (50µg) of clotrimazole produced 17mm zone of inhibition against *Candida albicans.* Of all the zones produced, the extract of cola acuminata was comparatively superior except in the case of *Proteus mirabilis* in which the extract was completely impotent.

Minimum Inhibitory Concentration (MIC) of ethanolic extract of Cola acuminata of the Isolates.

The result obtained from minimum inhibitory concentration is presented in Table 3. The determined MIC for *Staph aureus* was *50mg*/ml and 25mg/ml for *Candida albicans. Proteus mirabilis* showed no form of susceptibility to the extract.

DISCUSSION

The study revealed that the seed extract of *Cola acuminata* contained bioactive agents known to be responsible for the antimicrobial properties of the seeds. These agents were alkaloids, tannins, flavonoids, cardiac glycosides and phlobatannins. This finding, is therefore, in conformity with the report of Sofowora (1986). The presence of these important pharmacologically active principles underscores why the seeds of Cola acuminata exhibited an inhibitory effect against almost all the clinical isolates tested. Irvine, (1961) and Burkill, (1995) independently recognized that *C. acuminata* inhibited the growth of test organisms in urinary tract infections and gastroenteritis in man, *Cola acuminata* has traditionally been recognized in several tribal groups of Nigeria as the treatment for diarrhea, dysentery, cough and vomiting. The findings in this study may justify some of these traditional uses.

The finding in this study, of a high level of susceptibility of *Staphylococcus aureus and Candida albicans* to ethanolic extract of the seeds of *C. acuminata* may be in line with earlier reports of Ebana *et al*., (1991) and Reid *et al*., (2005) on some species of Cola. It is, however, in total contrast to some related research on the leave of the cola. Some studies on antimicrobial activities of the leaves of *C. acuminata* revealed their inability to inhibit *Staphylococcus aureus*, *Staphylococcus albus, Klebsiella pneumonia,* and *C. albicans.* In this study, the leaf *C. acuminata* was not investigated. But, Aladesanmi *et al*., (2007) had observed that the seeds *C. acuminata* had a greater potent activity than its leaves. However, the finding that 400mg/ml concentrations of the seed extract of cola acuminata exhibitory a superior antimicrobial activity against *S. aureus* and *Candida albicans* as compared to the drugs evaluated may justify the wide spread use of cola acuminata in traditional settings for the treatment of various forms of infections.

In this study, the antifungal activities exhibited by the extract of the seed of *Cola acuminata* were stronger and more pronounced than its antibacterial activities. *Cola acuminata* showed high inhibitory activity against *C. albicans* at different concentrations of 25mglml, 50mglml, 200mglml and 400mglml with a comparatively higher zone of inhibition. The reasons for this disparity may not be

sufficiently explained, however, studies have shown that antimicrobial activity may involve complex mechanisms membrane, nucleic acids and protein as well as the inhibition of the metabolism of nucleic acids (Oyaizu et *al*., 2003), investigations which were all outside the scope of this work.

Furthermore, the low minimum inhibitory concentrations (MIC) demonstrated by the extract against *Staphylococcus aureus* and C*. albicans* further add to reveal its highest antimicrobial potentials. The finding of a high level of resistance of *Proteus* species to all levels of concentration of the extract reveals the limitation in the use of the extract. A study involving the use of a broader group of gram negative organisms may be necessary to conclusively evaluate the antimicrobial potentials of the seeds of *Cola acuminata.*

CONCLUSION

This study shows the presence in the extract of *Cola acuminata* seed of five major components Alkaloids (moderate), and abundant presence of Tannins, Cardiac glycosides, flavonoids and Phlobatanins and total absence of Saponins. It also reveal that ethanolic extract of Cola acuminata is a superior antimicrobial agent against *Staphylococcus aureus* and *Candida albicans*, but not for *Proteus mirabilis* as compared to the drugs evaluated.

REFERENCES

Aladesanmi AJ, Iwalewa EO, Adebayo AC, Akinkunmi EO, Taiwo BJ, Olorunmola FO, Lamikanra A (2007). Antimicrobial and Antioxidant activities of some Nigerian Medicinal Plants. *Afri. J. Trad*. CAM. 4(2):173-184.

Ayensu ES (1978). Medicinal Plants of West Africa. Reference publication International, Michigan, Michigan.P.78.

Ayodele EA (1995). nPhysico-Chemical Properties of some cola growing soils of Nigeria. *Nigerian Journal of Tree Crop Research* 1(2):37-51.

Burkill HM (1995). The useful plants of West Tropical Africa. 2nd Edition.II.Vol.3.Botanical Garden. Pp. 522-527.

Cheesebrough M (2001). *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press, Uk. Pp.137-139.

Clinical Laboratory Institute Standards (CLIS) (2005). Performance standards for antimicrobial susceptibility. 15th informational supplement (CLIS document M100 –S15). Vol. 25, Wayne, P.A.

Ebana RUB, Ekpe BE, Madunagu BE, Otung IN (1991). Microbiological Exploitation of Cardiac Glycosides and Alkaloids from Garcinia kola, Borreria Ocymoides, Kola nitida and Citrus aurantifolia. *J. Appl. Bacteriol*.71:393-401.

Hirt HM, `pia BM (2001). Natural Medicine in the Tropics. Anamed. 2nd Edition; Drucherei Bauer, Winnenden. Pp.187-189.

Iroha IR, Oji AE, Afiukwa TN, Nwuzo AC (2008). Antimicrobial Activity of Extracts of Garcinia kola Against Resistant Extended Spectrum Beta Lactamase producing *Escherichia coli* and *Klebsiella pneumonia. Nig. J. Microbiol*. 22 (1):1698-197.

Irvine FR (1961). *Wood Plants of the Ghana.* Vol. 17. Oxford University Press. Pp. 498-502.

Iyere IJ (2011). The Socio - Religious Significance of Obi (Kola Nut) among the Igala People of Kogi State Cross-Cultural Communication 7(2):199-208

Kiple KE, Ornelas KC (2000). Cola nuts *In*:*The Cambridge World History of Food*, Cambridge University Press. New York, NY.Pp.684-692.

Kubata BK, Nagamune K, Muramkami N, Merkel P, Kabututu Z, Samuel K, Martin M,

Nickalls RWD (1986). W.F. Daniel. (1917-1965).The discovery that the cola nuts contain caffeine. *The Pharmaceutical Journals*.236:401-402.

Odugbemi T (2006).*Outlines and Pictures of Medicinal Plants from Nigeria.* University of Lagos Press. P.158.

Oyaizu MY, Fujimoto H, Ogihara K, Sekimoto A, Naruse U (2003). Antioxidative and Antimicrobial activities of extracts from several utility plants. *Food Preservative Science*.29:33-38.

Rätsch C (2005). The Encyclopedia of Psychoactive Plants: Ethno-pharmacology and Its Applications .Park Street Press, USA; pp657-7.

Reid KA, Jager AK, Light ME, Mulholland DA, Staden JV (2005). Phytochemical and Pharmacological Screening of *Sterculiaceae* species and Isolation of antibacterial Compounds. *J. Ethnopharmacol*. 97 (2):285-291.

Sofowora A (1993). Medicinal Plant and Traditional Medicine in Africa. 2nd Edn., John Wiley and Sons, New York., USA., pp: 6-65.

Sundstrom L (1966). The Cola nuts. Function in West Africa social Life. Studia Ethnopgraphia Upsaliensia, 26:135-145.

Taba K, Mustakuk H, Yoshida M, Ohshishi M, Kameyama KT, Duszenk M, Urade Y (2005). Cola acuminata Proantho Cyanidins: a Class of antitrypansomal compounds effective against *Trypanosoma bruci*. *Inter. J. parasitol*. 35:91-103.

Trease GE, Evans WC (1989).Trease and Evans Pharmacognosy.13th Edition.London: Bailliere Tindale.P.832.