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Phytochemical Analysis and Antimicrobial Studies of Different Extracts of Cola Nitida and Cola Acuminata Sourced from Different States in Southern Nigeria

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Abstract: Many Nigerians consume kola nuts regular, even daily, for cultural, medicinal and as a stimulant against sleep, among many other reasons. Some people consume kola nuts to treat fungal, bacterial, viral and parasitic infections such as typhoid, cough, pneumonia, skin and tooth infections. This study investigates the phytochemical composition of extracts of kola nuts from different parts of Southern Nigeria, assessed the antimicrobial activities of Cola acuminata and Cola nitida extracts and compared the antibacterial activities of kola nut extracts and synthetic antibiotics. The seeds of the kola nut were obtained from farmers in Udi, Ikom and Ado-Ekiti in Nigeria. Kola nuts were identified in the herbarium department of Plant Science and Biotechnology, University of Nigeria Nsukka. Kola nuts were shield dried, and manually cut to smaller sizes, grinded and prepared for analysis. Three bacterial isolates such as Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli were used for the study. The phytochemical compositions of kola nuts were determined. Qualitative and quantitative phytochemical studies showed that terpenoid was the highest, followed by caffeine. Antibacterial studies showed that Ciprofloxacin and Pefloxacin have strong antibacterial activity across all tested bacteria. ($p<0.05$). This study demonstrated that the methanolic and acetonic extract of Cola acuminata and Cola nitida showed significant antibacterial activity against most of the selected bacterial isolates. However, the effect is not comparable with standard antibiotics (ciprofloxacin, pefloxacin, and augmentin).

Key words: Cola acuminata, Cola nitida, extracts, phytochemical, antimicrobials

Introduction

Background The use of herbal produce in the treatment of various ailments continue to receive worldwide attention by researches as traditional medicines derived from plants have become highly important as alternative medicines in treatment of a huge number of diseases. These plants derivatives are considered safe and more affordable for majority of the developing world as it is used for the treatment of pathogenic infections especially difficult to treat bacterial infections which are resistant to modern synthetic antibiotics.

Cola nitida has two lobes which have pink and milk colour. It is cheaper than Cola acuminata. Cola nitida cultivated in southern Nigeria. Those that were cultivated in south west and south south Nigeria appears milk in colour while those that were cultivated in south east Nigeria appears pink in colour. Moreover, Cola acuminata is pinkish in colour and has more than two lobes which range from three to ten lobes. It is used in Udi and other parts of south east Nigeria for welcoming visitors, rituals, traditional ceremonies and peacemaking (Odo et al. 2023).

Figure 1.0 Cola nitida from Ado-Ekiti with two cotyledons (left), Cola acuminata (middle) Ikom and Cola acuminata from Udi with more than two cotyledons (right).

Cola acuminata and Cola nitida has been reported to have antimicrobial activities against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (Afolabi et al 2020; Iteku 2021; Nwomuma et al 2023).Traditional practitioners have used kola nuts for the treatment of microbial infections including typhoid fever, eye infections, urinary tract infections, and many other bacterial and fungal infections. Many northerners in Nigeria habitually eat some types of kola nuts which they believe protects, strengthens and heals the teeth from all dental problems and ailments.

Kola nuts are common sight in Nigerian markets, cities and villages. They are often sold by street vendors at bus and train deports. Many Nigerians consume kola nuts regular, even daily, for cultural, medicinal and as a stimulant against sleep, among many other reasons. In traditional medicine, kola nuts are dried, grounded and mixed with honey to make a traditional cough mixture in rural areas in Udi Enugu state of Nigeria. However, some researchers have reported high concentration of caffeine in kola nut extracts which have detrimental effects on human health (Amadi and Nwachukwu 2020; Apiam et al 2023; Chukwuma et al 2023; Daniel et al 2024). Women in some parts of Nigeria chew kola nuts to prevent 'morning illness' association with early pregnancy. Many researchers have reported antimicrobial activities of Cola nitida and Cola acuminata (Afolabi et al 2020; Iteku 2021; Nwomuma et al 2023; Osadebe, 2021; Telles and Mekinsey 2020).

Material and Methods

Sample collection: One kilogram of seeds of the Cola nitida and Cola acuminata were obtained from farmers in Udi, Ikom and Ado-Ekiti in Nigeria between the months of April 2020 to November 2022. However, 500g of the kola nuts were used for antimicrobial and phytochemical studies.

Isolation of organisms: The sample size was six isolates of Escherichia coli, Staphylococcus aureus, and Pseudomonas auruginonsa including clinical and environmental organisms. The clinical isolates were collected from Medical Laboratory Department (Medical Microbiology Unit) National Orthopaedic Hospital Enugu State. The environmental organisms were isolated from decaying food sources such as Pork meat, yoghurts, maize, cashew nuts, beans and lots more. Moreover, the kola nut extracts activities were compared with antibiotics (ciprofloxacin, pefloxacin and augmentin). The micro organisms were identified by their Gram reactions, cultural characteristics and biochemical activities using standard bacteriological methods.

Identification of Kola nuts and sample preparation for analysis:

Samples of each kola were identified by a taxonomist at the department of Plant Science and Biotechnology, University of Nigeria Nsukka. A voucher specimen (UNH NO. 1836[5]) was deposited at their herbarium for future references. Sample was shield dried and manually cut to smaller sizes. After drying, sample was grinded in laboratory blender, extracted with the help of soxhlet extractor. The product was sealed and stored in cool, dry condition for the analysis. .

Phytochemical analysis was done to determine the presence and quantity of theobromine, phenols, anthraquinone, steroids, flavonoids, alkaloids, saponins, cardiac glycosides, tannins and terpenoids (Chukwuma et al. 2023).
Data Analysis: Data analysis was done with SPSS version 25. Quantitative variables were presented with mean standard deviation and student t-test at P<0.05.

Results

Table 4.0 Qualitative phytochemical analysis of methanol extract of Cola nitida and Cola acuminata from different states in southern Nigeria. From the table above, terpenoid was the highest, followed by caffeine, and the least was anthraquinone.

Methanol extracts

(+) Present, (++) Moderate, (+++) High, (++++) Very High

Sample %	Alkaloid %	Saponin %	Tannin %	Caffeine %	Glycoside %	Terpenoid %	Phenol %	Anthraquine %
Cola nitida (Udi, Enugu)	+	+	+	++	+	++++	+	_
Cola nitida (Ado Ekiti)	+	+	+	+++	+	++++	+	+
Cola nitida (Ikom Cross River)	++	+	+	+++	+	++++	+	+
Cola acuminata (Udi Enugu)	++	+	+	+++	+	++++	+	_
Cola acuminata (Ado Ekiti)	+	+	+	+++	+	++++	+	+
Cola acuminata (Ikom Cross	+	+	+	+++	+	++++	+	+

Table 4.1 Quantitative phytochemical analysis of methanol extracts of Cola nitida and Cola acuminata from different states in southern Nigeria. Cola acuminata from Ikom Cross River contained highest 7.90% of terpenoid while the least was anthroquinone from all zones.

Sample %	Alkaloid %	Saponin %	Tannin %	Caffeine %	Glycoside %	Terpenoid %	Phenol %	Anthraquine %
Cola nitida (Udi, Enugu)	0.62	0.29	0.022	1.1	0.34	4.89	0.021	0
Cola nitida (Ado Ekiti)	0.41	0.46	0.021	2.1	0.75	7.42	0.019	0.004
Cola nitida (Ikom Cross River)	1.39	0.34	0.023	2.6	0.49	5.25	0.026	0.002
Cola acuminata (Udi Enugu)	1.45	0.46	0.024	1.9	0.64	4.89	0.017	0
Cola acuminata (Ado Ekiti)	1.01	0.52	0.023	2.5	1.04	6.75	0.017	0.006
Cola acuminata (Ikom Cross	0.87	0.44	0.025	1.9	0.78	7.9	0.02	0.004

Table 4.2 Comparison of antibacterial activity of two kola species (Cola nitida and Cola acuminata) and ciprofloxacin against bacteria isolated from Udi. The analysis utilized the Mann-Whitney U test with a significance level of 0.05. Ciprofloxacin shows strong antibacterial activity across all tested bacteria, both environmental and clinical, with consistent inhibition zones with statistically significant difference ($p<0.05$).

Cola nitida					
		Ciprofloxacin	**Acetone extract**	**Ciprofloxacin**	**Methanol extract**
Environmental bacteria	**S. aureus**	34	0	34	8.3
	E. coli	28	0	28	0
	P. aeruginosa	21	0	21	0
P- value		**0.002**		**0.006**	
Clinical bacteria	**S. aureus**	31	14.8	31	0
	E. coli	31	0	31	0
	P. aeruginosa	30	3.8	30	3.8
P-value		**0.005**		**0.000**	
Cola acuminata					

		Ciprofloxacin	Acetone extract	Ciprofloxacin	Methanol extract
Environmental bacteria	**S. aureus**	34	4.3	34	11.7
	E. coli	28	6.0	28	0
	P. aeruginosa	21	17.9	21	14.8
P- value		**0.03**		**0.03**	
Ki Clinical bacteria	**S. aureus**	31	12.7	31	16.2
	E. coli	31	0	31	0
	P. aeruginosa	30	15.7	30	14.8
P-value		**0.01**		**0.017**	

Table 4.3 Comparison of antibacterial activity of Cola nitida and Cola acuminata extracts (acetone and methanol) with Ciprofloxacin against bacteria from Ikom. The analysis utilized the Mann-Whitney U test with a significance level of 0.05. Ciprofloxacin shows high activity across all bacteria. ($p < 0.05$).

Cola nitida					
		Ciprofloxacin	**Acetone extract**	**Ciprofloxacin**	**Methanol extract**
Environmental bacteria Ikom	**S. aureus**	34	10.3	34	4.6
	E. coli	28	5.7	28	10.1
	P. aeruginosa	21	0	21	7.42
P- value		**0.009**		**0.007**	
Clinical bacteria Ikom	**S. aureus**	31	7.2	31	4.6
	E. coli	31	7.2	31	10.6
	P. aeruginosa	30	4.3	30	7.2
P-value		**0.000**		**0.0002**	
Cola acuminata					
		Ciprofloxacin	**Acetone extract**	**Ciprofloxacin**	**Methanol extract**
Environmental bacteria	**S. aureus**	34	8.5	34	5.2
	E. coli	28	3.7	28	4.6

Ikom	**P. aeruginosa**	21	6.2	21	4.0
P- value		**0.006**		**0.004**	
Clinical bacteria Ikom	**S. aureus**	31	8.5	31	5.2
	E. coli	31	3.7	31	4.6
	P. aeruginosa	30	6.2	30	4.0
P-value		**0.000**		**0.000**	

Table 4.4 Comparison of antibacterial activity of Cola nitida and Cola acuminata extracts with Ciprofloxacin against bacteria from Ado-Ekiti. The analysis utilized the Mann-Whitney U test with a significance level of 0.05. Ciprofloxacin consistently shows high activity across all bacteria. ($p < 0.05$).

Cola nitida					
		Ciprofloxacin	**Acetone extract**	**Ciprofloxacin**	**Methanol extract**
Environmental bacteria	**S. aureus**	34	10.5	34	3.8
	E. coli	28	4	28	7.3
	P. aeruginosa	21	0	21	0
P- value		**0.009**		**0.005**	
Clinical bacteria	**S. aureus**	31	10.7	31	4.7
	E. coli	31	8	31	9.7
	P. aeruginosa	30	2.4	30	5.8
P-value		**0.0007**		**0.0001**	
Cola acuminata					
		Ciprofloxacin	**Acetone extract**	**Ciprofloxacin**	**Methanol extract**
Environmental bacteria	**S. aureus**	34	5.5	34	3.3
	E. coli	28	5.8	28	2.5
	P. aeruginosa	21	6.9	21	6.3

P- value		**0.005**		**0.004**	
Clinical bacteria	**S. aureus**	31	0	31	1.7
	E. coli	31	2.5	31	1.7
	P. aeruginosa	30	9.1	30	7.4
P-value		**0.0006**		**0.0002**	

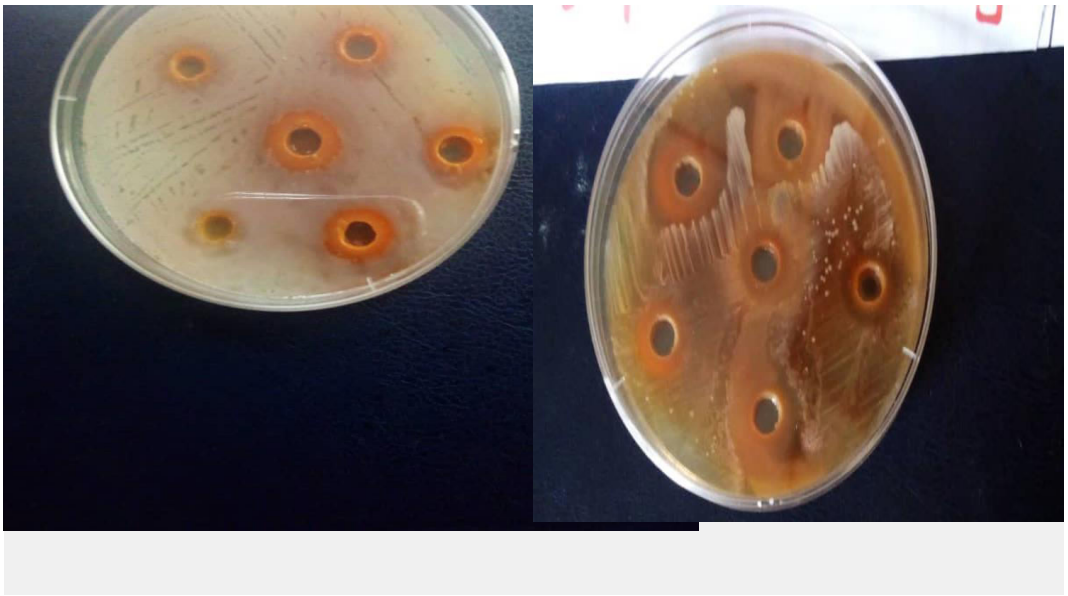

Figure 4.1 Antibacterial activities of Cola nitida and Cola acuminata extracts on environmental isolates of Staphylococcus aureus.

Discussion

The quantitative phytochemical analysis of this study showed that Terpenoid was the highest, followed by Caffeine and the least was Anthraquinine. Cola acuminata extract from Ikom Cross River State contained the highest percentage of Terpenoid (7.90%), Cola nitida extract from Ikom Cross River State contained the highest percentage of Caffeine (2.60%) which when consume in excess has detrimental effects (Egoro et al. 2023; Adelusi et al. 2020; Mbembo et al. 2021). Cola acuminata extract from Udi Enugu State contained the highest percentage of Alkaloid (1.45%) from this study which correlates with studies done by other researchers (Amadi and Nwachukwu 2020; Apiam et al. 2023; Chukwuma et al. 2023; Daniel et al. 2024; Mbembo et al. 2021; Nwomuma et al. 2023). Antimicrobial studies of methanolic and acetonic extracts of Cola acuminata and Cola nitida on selected bacteria which

included Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, which were isolated from environment and clinics showed variable zone of inhibition diameter at different concentrations of the extracts which concurred with other literatures (Afolabi et al 2020; Iteku, 2021)

The study showed that Cola acuminata (methanol extract) from Udi Enugu State has the highest zone of inhibition 16.166mm on clinical isolate of Staphylococcus aureus. However, acetone and methanol extracts of Cola nitida from Ikom has the highest zone of inhibition of 10.83mm and 10.33mm on Escherichia coli (clinical) when compared with antibacterial activities of extracts from other zones. Acetone and methanol extracts of Cola acuminata from Udi showed highest zone of inhibition on clinical isolates of Pseudomonas aeruginosa, this conforms with other studies (Nwomuma et al. 2019; Osadebe, 2021; Telles and Mekinsey, 2020; Ugwugwo et al. 2020)

Antibiotics used in this study (Ciprofloxacin and Pefloxacin) has high zone of inhibition diameter when compared with the methanolic and acetonic extracts of Cola acuminata and Cola nitida which have low ZID with statistically significant difference ($p<0.5$).

Conclusion

This study demonstrated that the methanolic and acetonic extract of Cola acuminata and Cola nitida showed significant antibacterial activity against most of the selected bacterial isolates. However, the effect is not comparable with standard antibiotics (ciprofloxacin, pefloxacin, and augmentin).

Conflict of interest: This study is declared no conflict of interest

References

1. Afolabi B. T., Agu G. C and Onajobi I. B. (2020). Phytochemical screening and antimicrobial activity of Garcinia kola (Hackel) and Cola nitida (vent) extracts. Nigerian Journal of Technology. 39 (2) 379-385. Pharmaceutical Sciences.
2. Adelusi A. A., Ogunwolu Q. A., Ugwu C. A., Alli M. A., Adesanya K. A., Agboola M. O. and Akinpelu A. O. (2020). Kolanut consumption, its benefits, and side effects. World Journal of Advance Research Review. 8(3):356-362.
3. Amadi C. N., and Nwachukwu W. I. (2020). The effects of oral administration of Cola nitida on the pharmacokinetic profile of metodopamine in rabbits. BMC Pharmacology and Toxicology.
4. Apiam A., Orhonigbe I., Evuen U. F., Kadiri H. E., Okoro I. O., and Kpoma E. D. (2023). Invitro nutitional, phytochemical, antioxidant and anti-inflammatory analysis

indicated disparity in Cola nitida Garcinia kola Hekel used in Nigeria. FUDMA Journal of Sciences 7(3):102-111.

5. Chukwuma I. F., Apeh V. O., Nwora F. N., Nkwocha C. C., Mba S. E., and Ossai E. C. (2023). Phytochemical profiling and antioxidative potential of phenolic-rich/ extracts of Cola acuminata nuts. Biointerface Research in Applied Chemistry 13(1):29.
6. Daniel E. J., Imaobong U. N., and Sylvester C. (2024). Cola acuminata: nphytochemical constituents, nutritional characteristics, scientific validated pharmacological properties, ethnomedicinal uses, safety considerations, and commercial values. Herbal Medicine Phytochemistry. Pp 1-39.
7. Egoro E. T., Oni E. M. and Odumoson C. N. (2023). Assessment of consumption of kola nut (Cola nitida) on hepato-renal and reproductive hormones biomarkers among chronic consumers in Aritallin Bayelsa State Nigeria. World Journal of Biology Pharmacy and Health Sciences 14(01):216-221.
8. Iteku J. B., Bongo G. N., Mbiya J. N., Wambala J. M., Mutwale P. K., Inkoto C. L., and Ngbolua K. N. (2021). Phytochemical analysis and evaluation of bioactivities of Cola acuminata extracts. Journal of Biomedical and Life Sciences, 1(1):51-65.
9. Mbembo B. M., Inkoto C. L., Amogu J. J. O., Ashande C. M., Kutshi N. N., Nagahuedi J. M. S., Mpiana P. T. And Ngbolua K. N. (2021). Mini-review on the phytochemistry, pharmacology and toxicology of Cola nitida (Vent) Schott and Endl (Malvaceae): a medically interesting bio-resource of multiple purposes in Africa. Discovery Phytomedicine 8(4): 356-362.
10. Nwomuma C. O., Adelani-Akande T. A., Osemwegie O. O., Olaniran A. F. And Adeyomo T. A. (2019). Comparative Study of the in-vitro phytochemicals and antimicrobial potential of six medicinal plants. F1000 Res. 8:81.
11. Odo O. N., Nwokeocha I., and Ezegwu D. (2023). 'He who brings kola brings life' : Communicating the significance of kola nuts among Igbo people of Nigeria. India Journal of Arts Literature 4(1):24-29.
12. Osadebe P. O. (2021). An investigation into the seasonal variation of the phytochemical and anti-diabetic properties of the eastern Nigerian specie of African mistletoe and Kola acuminata. Diabetes Complications 5(2):1-5.
13. Telles C. and Mckinsey C. (2020). The efficacy of Cola acuminata on gram-negative bacteria. The FASEB Journal 34(1):1-1.
14. Ugwugwo B. O., Ahmed A., Oluwasola H. O. and Ukoha P. O. (2021). Comparative assessment of phytochemicals, antioxidant activity and antimicrobial activity of Cola acuminata, Garcinia kola and Vernonia amygdalina. Journal of Chemical Society of Nigeria 46(4):0698-0710.