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Phytochemical Screening and Antimicrobial Evaluation of Ethanol Extract of *Cola lepidota* Seeds

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Authors' contributions

This work was carried out in collaboration between both authors. Authors EJU and IOA the two authors were actively involved in all stages of this manuscript: The study design, laboratory analysis, statistical analysis, sourcing for literatures and writing of manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was carried out to examine the antimicrobial activity of ethanol extract of *Cola lepidota* seeds.
Place and Duration of the Study: Samples for the study; *Cola lepidota* seeds were collected from Nto Unang Atan in Ekpenyong Atai 1, Essien Udim L.G.A., Akwa Ibom State. The samples were analyzed in the Chemistry laboratory of Akwa Ibom State University and Microbiology laboratory of University of Uyo, Akwa Ibom State, Nigeria. The work lasted for three months.
Methodology: Air-dried seeds were ground to fine powder using a manual grinding machine and were stored in an airtight container until further use. The ground sample (20 g) was extracted with 80 mL of ethanol at room temperature for 72 hours using maceration method. A qualitative phytochemical test was conducted on the ethanol extract of the seeds of monkey cola. The antimicrobial activity of the extract was evaluated for six bacteria and four fungi using the well in agar diffusion technique.

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Results: The result of the phytochemical screening revealed the presence of all the phytochemical tested except tannins, anthraquinone, and resins. The moisture content and dry weight matter of the seeds were determined to be 35.87 ± 0.77% and 64.13 ± 0.77% respectively. All the pathogenic bacteria and fungi were found susceptible to the extract mostly within the extract concentrations of 400 mg/mL and 500 mg/mL; though some were inhibited at the lower concentrations of the extract. No activity was observed against *Pseudomonas aeruginosa* at all concentrations of the plant extract. Minimum inhibitory concentrations (MIC) of the extract were determined on *Staphylococcus aureus, Saccharomyces cerevisiae, Aspergillus flavus,* and *Aspergillus niger* to be 100, 350, 150, and 150 mg/mL respectively.
Conclusion: The bioactivity of the ethanol extract of *Cola lepidota* seeds may be attributed to the interaction among the phytochemicals present in the extract. *Cola lepidota* seeds that seem to be treated as waste may be harnessed into pharmaceuticals for healthcare maintenance.

Keywords: Concentrations; maceration; pathogens; phytochemicals; susceptibility.

1. INTRODUCTION

"Monkey kola is an African indigenous fruit that is popularly grown. It is a specie of the West African kola nut tree. The scientific name of monkey kola is *Cola lepidota K. Schum*. It is widely cultivated in many parts of Nigeria. Monkey kola is a common name used to identify some minor relatives of the Cola species that produce ripe juicy fruits" [1.2]. "Although the common name of the fruit is monkey kola, it is known as Ochiricha or Achicha in Igbo, Obi Edun in Yoruba, Goron or birri in Hausa, and Ndiyah in Ibibio or Efik. Yellow monkey kola fruit (*Cola lepidota*) is mostly consumed by native people of southern Nigeria and the Cameron, as well as some wild primate animals especially monkeys, baboons, and other species" [2,3.4]. "The seeds of the monkey cola species are obliquely ovoid with two flattened surfaces, rough and reddish brown or green; but not edible unlike the seeds of cola nut (*Cola. nitida*). There are three main species of monkey kola: yellow, white, and red monkey kola. The yellow monkey kola is the most popularly known among the three categories. The lack of good database and poor knowledge of the nutrient composition and quality of traditional food crops are some of the reasons for low fruit and vegetable consumption in developing countries" [5]. Tropical African sub-regions are home to many valuable fruit species whose potentials have not been fully realized. Most of them have not been identified and evaluated for their nutritional and functional properties and therefore are underexploited. One of such plant foods is *Cola lepidota* (Monkey kola). The fruit is a good source of crude protein, fibre and fat, Ca, Mg, Zn, Cu, β-carotene and niacin, while the pulp is a good source of ash, starch, carbohydrate, K, P and Se contents [6]. The pulp (mesocarp) is the most commonly consumed part of this fruit. The seeds of *C. lepidota* is reported to be employed in Nigerian folk medicine for pulmonary problems and cancer related ailments. In Nigeria about twenty-three species are known and some are used in traditional medicine as stimulant, to prevent dysentery, and to suppress sleep [7]. The medicinal importance of the seed of monkey kola (*Cola lepidota*) is based mainly on the phytochemical components of the plants such as tannins, terpenoids, flavonoids, phenols, coumarins, and anthocyanins [8], out of which flavonoids have been observed earlier to be the most abundant [9]. The high fiber and low carbohydrate obtained in Monkey kola can play a key role in the diets of diabetic and hypertensive patients [9]. Monkey kola seed has been reported to have substantial amounts of minerals like iron, zinc, and copper; B vitamins, and vitamin C [9]. The seed of yellow monkey kola fruit (*Cola lepidota*) has been observed to have hypolipidemic effects [10]. There is scanty research and information on the medicinal values of monkey cola species particularly *Cola lepidota* seeds. In 2007, the World Health Organization expressed ongoing interest in the application of medicinal plants to treat various illnesses [11]. Thus, exploring and understanding the phytochemical composition and antioxidant potential of non-conventional plants may encourage utilization of these plants for nutraceutical and pharmaceutical purposes. It is therefore the aim of this study to evaluate the phytochemicals and antimicrobial activity of ethanol extract of *Cola lepidota* seeds. *Cola lepitoda* seeds that seem to be treated as waste may be harnessed into pharmaceuticals for healthcare maintenance.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The monkey cola (*Cola lepidota*) seeds were collected from Nto Unang Atan in Ekpenyong Atai 1, Essien Udim L.G.A., Akwa Ibom State. *Cola lepidota* seeds collected were washed and air-dried at room temperature of 23 - 29 ^{o}C for 15 days. The dried seeds were ground with a grinder to fine powder and stored in an airtight container until further use. Ground sample (20 g) was extracted with 80 mL of ethanol at room temperature for 72 hours using maceration method. After 72 hours, the extract was filtered with Whatman filter paper and concentrated using rotary evaporator.

2.2 Determination of Moisture Content of *Cola lepidota* Seeds

Determination of moisture content of the fresh seeds of *Cola lepitoda* was carried out for three consecutive time using moisture analyzer, model XY-105MW and the results obtained were as reported in Table 2.

2.3 Phytochemical Assay of the Ethanol Extract of *Cola lepidota* Seeds

Concentrated ethanol extract was used for phytochemical screening according to the methods prescribed by Rangari [12] in Table 1.

2.4 Antimicrobial Assay of Ethanol Extract of *Cola lepidota* Seeds

(i) Collection of microbial test organisms

Isolates were obtained from the Microbiology Laboratories of University of Uyo, as well as the the University Health Centre and University of Uyo Teaching Hospital, all in Uyo, Nigeria. The organisms included: *Staphylococcus aureus, Salmonella spp., Shigella spp., Escherichia coli, Pseudomonas aeruginosa, Vibro cholerae, Candida* albicans, *Saccharomyces cerevisiae, Aspergillus niger, Aspergillus flavus.* These organisms were sub-cultured and preserved as pure cultures on Nutrient agar and Sabouraud Dextrose agar slants and stored at low temperatures until required.

(ii) Preparation of test organisms used for the work

The organisms were diluted using 10-fold dilution to 10^{-3} for G+ve and fungi, 10^{-5} for G-ve thereafter the turbidity of the broths were compared with MacFaland standard [13].

(iii) Determination of extract concentrations

The extracts were dissolved using sterile water to constitute different concentrations of 500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL and 100 mg/mL.

(iv) Determination of antimicrobial assay of extract on selected test organisms

Antimicrobial activity of the extracts was evaluated using the well in agar diffusion technique [14]. The diluted test organisms on Nutrient broth and Malt Extract broth for bacterial and fungal isolates respectively were further sub-cultured to Peptone water and cells adjusted to MaFaland Turbidity standard. 0.1 mL of each diluted test organisms were aseptically transferred and spread on the surface of the Muller Hinton agar (MHA). Sterile swab sticks were used to spread the inocula on the surface of the medium and allowed them to dry on the bench. A sterile cork borer of 5 mm was used to bore holes on the surface of the medium that were seeded with the test organisms. In each of the wells previously seeded with the test organisms, 100 ug of the extract dilution of different concentrations were introduced into the wells.

Control experiments were set up alongside with the extracts using commercial antibiotics and antifungal drugs (Gentamycin and Nystatin); 20 mg and 50 mg for bacterial and fungal respectively. All plates were left on the bench for 1 hour before incubating at 37 ^{0}C for 24 hours. for bacterial and 72 hours for fungal isolates. After incubation, antimicrobial activities were determined by measuring the Zones of inhibition diameter (ZID) in all the extracts.

(V) Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the agar plates method. To obtain this, 1.0 mL from the last concentration of the extract that had activity for an organism were further diluted. 1.0 mL from each diluted extract were introduced into sterile plates. Nutrient agar (NA) and Sabouraud Dextrose agar (SDA) were poured into those plates, swirled and left on the bench to set. These were used for bacteria and fungi isolates

respectively. The plates were dried at low temperature and organisms that were susceptible to the extracts were inoculated on the plates by streaking method and incubated for 24 hours for bacteria and 48 hours for fungi. The concentration that inhibits the growth of any test organisms were noted as MIC for the extract on the test organism [13].

Table 1. Phytochemical screening test of ethanol extract of *Cola lepidota* seeds

Chemical groups	Chemical test	Result
Alkaloids	**Dragendroff's test**: 1.0 ml of extract + 1.0 ml of Dragendroff's reagent	Yellow or orange precipitate
	Wagner test: 1.0 ml of extract + 1.0 ml of wagner reagent	Brown-reddish precipitate
	Mayer test: 1.0 ml of extract + 1.0 ml of Mayer reagent	White or pale-yellow precipitate
Tannins	**Ferric chloride test**: Extract + 2ml ferric chloride solution.	Dark blue colour
Phlobatannins	**Acid test**: 3.0 ml of extract + 2.0 ml of 1% HCl + heat(optional)	Red coloured precipitate indicates the presence of phlobotannins
	Lead acetate test: 2.0 ml of extract + 3 drops of 5% lead acetate	A dark precipitate indicates the presence of phlobotannins
Carbohydrate	**Molisch test**: Extract + 1ml of Conc. H_2SO_4 acid	Violet ring formation indicates presence of carbohydrate
	Fehling solution test: Extract + fehling solution A & B + boiling on water bath	Brick red precipitate indicate the presence of reducing sugar.
Flavonoids	**Shinoda test**: Extract + magnessium turnings + 2 drops of conc. H_2SO_4	Pink colour indicates the presence of favonoid in the extract
	Alkali test: Extract + few drops of NaOH + dil. HCl	Yellow colour appears which turns colourless upon addition of dilute HCl.
	Zinc dust test: Extract + small amount of zinc dust + Conc. HCl	The solution turns red indicating the presence of flavonoids.
Saponin	**Foam test**: Extract is shaken vigorously for 2minutes. The foam (froth) + 3 drops of olive oil.	Stable foam formation indicates the presence of saponin. Formation of emulsion with olive oil further confirm the presence of saponin.
Cardiac Glycoside	**Liebermann's test**: Solution of extract in chloroform + equal amount of acetic anhydride + 2 drops of conc. H_2SO_4.	Formation of blue colour indicates the presence of cardiac glycosides
	Liebermann's Burchard test: extract + equal amount of acetic anhydride + few drops of conc. H_2SO_4 from the side of the test tube.	Red colour is first formed which changes to blue and then green.

Chemical groups	Chemical test	Result
	Kellers Killiani test (test for deoxysugars): 5 ml of extract + 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution + 1 ml of H_2SO_4 added slowly along the side.	A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.
	Salkowski test: Extract + few drops of conc. H_2SO_4.	Formation of violet colour indicates the presence of cardiac glycosides.
Steroids	Salkowski test: Extract + few drops of conc. H_2SO_4	At the lower layer steroid develop red colour
	Lieberman Burchard test: Extract + equal amount of acetic anhydride + few drops of conc. H_2SO_4 from the side of the test tube.	Steroid develop sap green colour
Anthraquinone	**Ammonia test**: Extract + ammonia Borntrager's test: chloroform extract of the plant sample + small volume of ammonia.	Pink or red colour indicate the presence of anthraquinone. Ammonical layer develops pink to red colour, indicating the presence of anthraquinone glycosides.
Polyphenol	**Nitric acid test**: Extract + dil. nitric acid.	Reddish - yellow colouration indicates the presence of polyphenol.
	Acetic acid test: Extract + dil. Acetic acid.	Red colouration indicates the presence of polyphenol
Triterpenoids	**Chloroform test:** Extract + 2ml of chloroform.	Formation of three layers indictes the presence of triterpenoid.
	Lieberman Burchard test: Extract + equal amount of acetic anhydride + few drops of conc. H_2SO_4 from the side of the test tube.	Triterpenoids gives a brown colour.
	Salkowski test: 5 ml + 2 ml of chloroform + 3 ml of conc. H_2SO_4 carefully added to form a layer.	A reddish-brown colouration of the inner face indicates a positive result for the presence of terpenoids.
Protein	**Biuret test**: Extract +Biuret reagent.	Purple or violet colour indicates presnce of protein.
	Xanthoproteic test: Extract+ nitric acid+sodium hydroxide.	Yellow or orange colouration indicates the presence of protein.
Resin	**Test for resin**: 0.2 g of the oil sample + 15 ml of 90% ethanol, then pour into 20 ml of distilled water the alcoholic extract.	A precipitate occurring indicates the presence of resins.

Table 2. Moisture content of fresh seeds of *Cola lepidota*

Initial Weight	Final Weight	Moisture Content (%)	Dry Weight (%)
1.510	0.980	35.180	64.820
1.110	0.720	35.400	64.600
1.310	0.825	37.030	62.970

3. RESULTS AND DISCUSSION

The result of the moisture content for *Cola lepidota* seeds is presented in Table 2. The percentage moisture content ranges from 35.18 to 37.03 %. This is relatively higher than 9.29% and 20.00% reported by Oranusi et al. [8] and [15] respectively as the percentage moisture content of the seeds of *Cola lepidota.* The moisture contents of *Cola lepidota* seed is low when compared to 62.50+0.08%w/w of *Cola nitida* (Red kola) and 63.80+0.08% w/w for *Cola acuminate* (white kola) by Etonihu et al. [16]. The moisture contents of food are usually used as a measure of stability and susceptibility to microbial [15]. The result of the antibacterial activity of the extract reported in Fig. 1, revealed that almost all the pathogenic bacteria used for the test were susceptible to the extract mostly at the concentration of 400 mg/mL and 500 mg/mL except *Staphylococcus aureus* that was susceptible to the extract at a lower extract concentration. *Pseudomonas aeruginosa* was resistant to the extract at all extract concentrations. Absence of antimicrobial activity of the ethanol extract of the seeds of *C. lepidota* against the tested pathogens at the extract concentration of 200 mg/mL and below was also reported by [8] except *Staphylococcus aureus* that was susceptible to the extract at the extract concentration of 200 mg/mL. In their studies, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were found resistant to the ethanolic extracts from endocarp, mesocarp and exocarp of the *C. lepidota* fruit within the concentration range of 25 to 200 mg/mL including the undiluted extracts. Comparison of the antibacterial activity of the extract with that of the standard antibiotic drug (Gentamycin) revealed exhibition of higher activity by the standard drug (at 20 mg/mL) than the extract (even at 500 mg/mL). The result of the antifungal activity of the ethanol extract of the seeds of *Cola lepidota.* (Fig. 2) showed that the zone of inhibition diameter (ZID) of the pathogenic fungi was extract concentration dependent with the highest activity observed against *Aspergillus niger.* Standard antifungal drug (Nystatin) used as control at the concentration of 50 mg/mL exhibited higher antifungal activity against all the tested pathogens than the ethanol extract of the seeds of *Cola lepidota.* The minimum inhibitory concentration of the extract on the tested pathogens were as reported in Table 3.

Table 3. Minimum inhibitory concentration (mic) of extracts on test organisms

Test Organisms	Extract Concentration (MG/ML)
Stapylococcus aureaus	100
Vibro cholerae	ND
Salmonella sp	ND
Shigella sp	ND
Escherichia coli	ND
Pseudomonas aeruginosa	ND
Aspergillus flavus	150
Candida albicans	ND
Aspergillus niger	150
Saccaromyces cerevisea	350

ND = Not determined

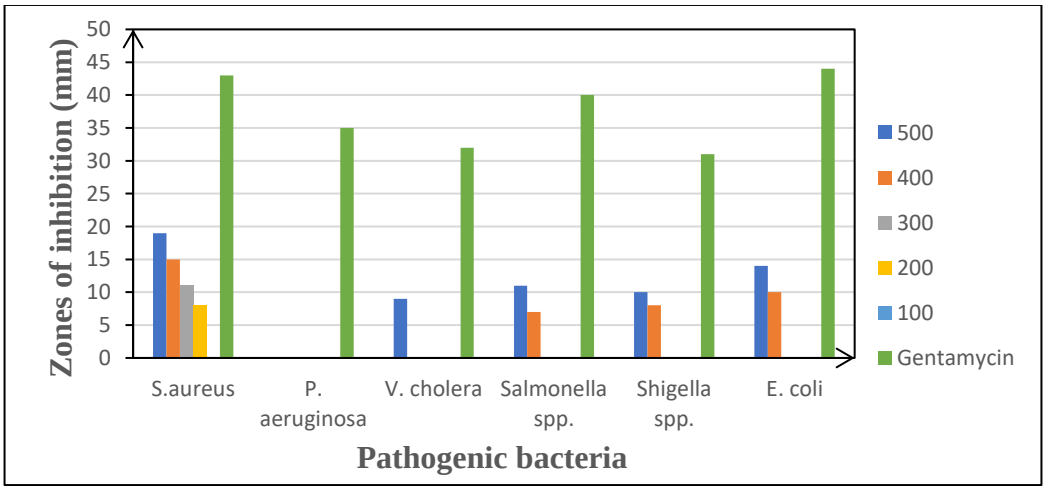

Fig. 1. Antibacterial activity of ethanol extract of seeds of *Cola lepidota*

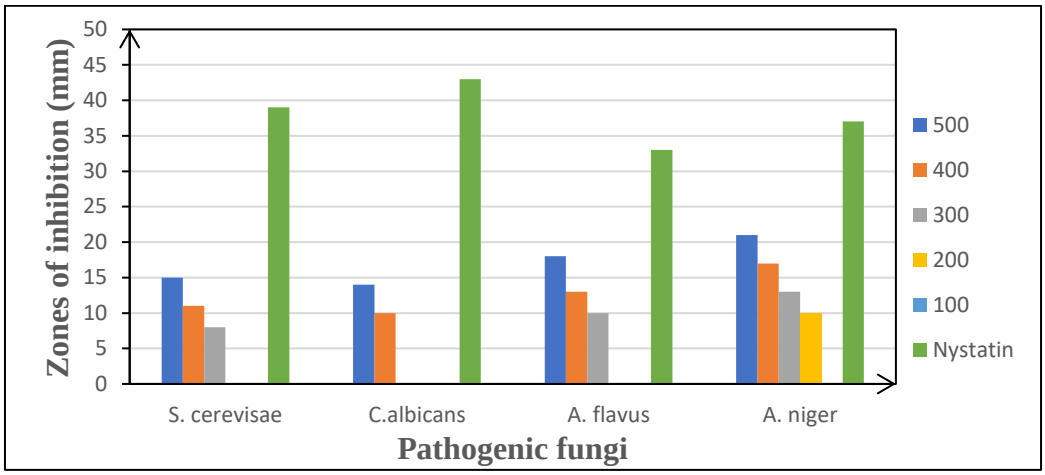

Fig. 2. Antifungal activity of ethanol extract of *Cola lepidota*

Table 4. Phytochemical screening of ethanol extract of seeds of *Cola lepidota*

Chemical groups	Chemical test	Results
Alkaloid	Dragendroff's test	++
	Wagner test	-
	Mayer test	+
Carbohydrate	Molish tet	+
	Fehling solution test	+
Tannins	Ferric chloride test	-
Phlobatannis	Acid Test	+
	Lead acetate test	+
Saponins	Foam Test	+
Flavonoid	Shinoda Test	+
	Alkali Test	++
	Zinc dust test	-
Cardiac Glycoside	Salkowski Test	-
	Lieberman's burchard Test	+
	Lieberman's Test	-
	Keller's Killian Test	-
Steroid	Lieberman's burchard Test	+
	Salkowski Test	+
Anthraquinone	Ammonia Test	-
	Borntrager's Test	-
Polyphenol	Nitric acid Test	++
	Acetic acid Test	-
Triterpenoids	Choloform Test	-
	Salkowski Test	++
Protein	Biuret test	+
	Xanthoproteic test	-
Resins		-

++ = high; + = low; - = absent

Of all the phytochemicals screened of the extract of seeds of *Cola lepidota* only tannins, anthraquinones and resins were absent. The presence of saponins, flavonoids, and phenols corroborates the studies of Oranusi et al. [8] who also reported the presence of these phytochemicals in the extract they obtained from *C. lepidota* seeds. The presence of some phytochemicals in *C. lepidota* seeds was also observed by Akpakpan et al. [17] in extracts from red and white kola nuts. The variability of phytochemicals in plants may be influenced by some primary factors such as genotype, size and maturity, soil conditions, fertilization, irrigation, pesticide utilization, disease and pests, location and climate, and season [18]. The observed antimicrobial activity possessed by the seed extract of *Cola lepidota* may be due to the presence of these phytochemicals [8,9,19]. Phenolics and flavonoids are known to be strong antioxidants and have anti-microbial, anti-inflammatory, anti-allergic, anti-mutagenic and anti-cancer activity and protect heart against diseases [20,21]. Though the mechanism of the activity by phytochemicals on bacteria is not fully understood, it is inferred that their antibacterial activity is through one of many mechanisms which include blockage of cell wall synthesis, inhibition of protein synthesis, disruption of nucleic acid biosynthesis, or lysis of microbial cells [22]. The antibacterial activity has been attributed to the presence of tannins, flavonoids, steroids, saponins, and alkaloids in the extract [19,23,24]. Saponins exhibit antioxidant and anti-inflammatory activity and are used in the management of hypercholesterolaemia and hyperglycaemia [25].

4. CONCLUSION

Cola lepidota seeds that seem to be treated as waste may be harnessed into pharmaceuticals for healthcare maintenance. The percentage moisture content ranges from 35.18 to 37.03 %. The ethanol extract of the seeds of *Cola lepidota* showed low antimicrobial activity against the tested pathogens at the extract concentration higher than 200 mg/mL. No activity was observed at lower extract concentrations. The bioactivity of the ethanol extract of *Cola lepidota* seeds may be attributed to the interaction among the phytochemicals present in the extract. Though absent of activity against some pathogens and generally low activity against most of them may be a display of antagonistic reaction among some of the phytochemicals in the extract. Further studies would be undertaken on the isolation of some bioactive ingredients from the seeds to see how medicinal properties of these seeds that had been treated as waste could be maximally developed and utilized.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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