

Comparative Preliminary Phytochemical Screening and Antibacterial Properties of the Fruit Epicarp and Seed Extracts of *Cola lepidota* K. Schum

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Abstract: In this study, comparative preliminary phytochemical screening and antibacterial properties of the fruit epicarp and seed extracts of *Cola lepidota* K. Schum against various bacterial strains with a view to ascertaining the scientific basis for its ethnobotanical use and establishing its effect was studied. The fruit of *Cola lepidota* K. Schum has been traditionally used in medicine for various therapeutic purposes. The extracts were obtained using methanol. The extracts were further analyzed for their phytochemical composition, including alkaloids, saponins, tannins, flavonoids, cardiac glycosides and anthraquinones. The antibacterial activities was evaluated using the agar well diffusion method and antibiotic susceptibility testing. The results showed that the seed extract exhibited more potent activity against *E. coli* (MIC of 0.25mg/mL) compared to the fruit epicarp extract. The seed extract also showed notable antibacterial activity against *Staphylococcus aureus*, *B. subtilis*, and *Pseudomonas* sp., with zone of inhibition values of 30.0mm, 20.0mm, 20.0mm, and 20.0mm, respectively, at a concentration of 100mg/mL. The fruit epicarp extract showed comparable antibacterial activity, although to a lesser extent. The high phytochemical potential in the extracts justify its folkloric use in the management, treatment of some degenerative diseases and as a source of novel antimicrobial agents.

Keywords: Phytochemical, Screening, Antibacterial, Extract, Inhibition

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1.0 Introduction

The emergence of antimicrobial resistance has become a significant global health concern, necessitating the search for novel antimicrobial agents. Plants have been a vital source of

medicinal compounds for centuries, with many modern medicines derived from plant extracts (Cragg *et al.*, 2013; Uffia *et al.*, 2024).

Since human existence, plants have been dependable sources of sustenance and medications to human beings. The plant parts and herbs from the olden time have been major sources of treatment for different health abnormalities in man and other living organisms (Udofia *et al.*, 2023a). However, due to modernization and the inability of native medical practitioners to give clean native treatment, there was a change in preference for medical treatment sources (Wangchuk, *et al.*, 2015; Uffia, *et al.*, 2025).

The use of plant extracts in traditional medicine is often based on empirical knowledge passed down through generations, with many plants being used to treat a range of ailments, including infections (Iwu, 2014).

The World Health Organization (WHO) data estimate that 80% of the global population primarily depends on traditional medicine for their primary healthcare needs. Their efficacy depends on diverse mechanisms triggered by plant extracts or their active components, increasing the interest in different medicinal plants. In Nigeria, traditional medicine plays a significant role in healthcare, with many people relying on plant-based remedies for their primary healthcare needs (African Health Monitor, 2003; WHO, 2019).

There is increasing interest in ingredients with potential use in precision medicine and shelf life extension. These interests are derivable due to the vast health-promoting benefits of plant bioactive contents. Plants and plant parts' bioactive compounds exhibit nutritive, health-promoting and preservative properties and are applicable as natural spices, natural preservatives and therapeutic agents against several diseases (Uffia *et al.*, 2021; Udofia *et al.*, 2023b).

Cola lepidota K. Schum, a plant species belonging to the family Malvaceae, has been traditionally used in Nigerian folk medicine for

various purposes, including the treatment of infections (Gill, 1992). The plant's seeds are commonly consumed for their stimulant properties, while the epicarp is often discarded. 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 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 (Okudu, 2015). The pulp (mesocarp) is the most commonly consumed part of this fruit.

The antimicrobial properties of plant extracts are attributed to the presence of various phytochemicals, including alkaloids, flavonoids, and terpenoids (Cowan, 1999, Okokon *et al.*, 2012). These compounds have been shown to exhibit antimicrobial activity against a range of microorganisms, including bacteria, fungi, and viruses.

There is limited scientific evidence on the antibacterial properties of this plant, particularly the fruit epicarp and great need for new antimicrobial agents to address the growing challenge of antimicrobial resistance. This necessitates the aim of this study to compare the phytochemical composition and antimicrobial properties of the epicarp and seed of *Cola lepidota* K. Schum.

Despite extensive ethnomedicinal use of *Cola lepidota* K. Schum, there is limited scientific evidence on its phytochemical profile and antibacterial activity. Existing studies are scarce, lack compound identification, and provide no standardized evaluation of its bioactive potential. This research addresses the gap by characterizing its chemical constituents and validating its therapeutic relevance.

This study provides new insight by identifying and characterizing bioactive compounds from *Cola lepidota* K. Schum, addressing the scarcity of scientific evidence supporting its ethnomedicinal use. The results bridge the knowledge gap on its phytochemical



composition and antibacterial potential, offering a scientific foundation for future drug discovery and therapeutic applications.

2.0 Materials and Methods

2.1 Plant collection and identification

Cola lepidota K. Schum was sourced from a local market in Uyo Local Government Area, Akwa Ibom State, Nigeria. The plant was identified by a taxonomist in the Department of pharmacognosy and natural medicine, University of Uyo and the Voucher number uuh815

2.2 Plant Extraction

The seeds and fruit epicarps of *Cola lepidota* K. Schum were washed properly to remove dirt and other contaminants, air dried and pulverized. Using a beam balance, 591g of each dried plant material was weighed before being transferred into a separate glass extraction jar containing 6.8L/ 680ml of 99.8% methanol respectively and kept at room temperature (25°C - 32°C) for 3days (72 hours) which was accompanied by periodic shaking. The extracts were filtered at the end of 72 hours and allowed to dry using a rotary evaporator at a temperature of 40° C. The concentrated methanol extracts was kept in a refrigerator at -4°C until further use.

2.3 Phytochemical Screening

Phytochemical screening was performed to identify bioactive chemical elements such as alkaloids, saponins, tannins, flavonoids, cardiac glycosides, and anthraquinones. The screening was done on each of the extract according to the standard methods. (Sofowora, 1993; Evans, 2002; AOAC., 2005; Prashant *et al.*, 2011)

2.4 Fractionation (Partitioning of Extract)

The extract (30g) was dissolved in 100ml of distilled water and partitioned successively and exhaustively with Hexane, DCM (Dichloromethane), Ethylacetate and Butanol using separating funnel to obtain their respective fractions. Subsequently, each

fraction was dehydrated under reduced pressure using a rotary evaporator (Lab Tech EV 311, China) operating at 35°C. The methanolic extract and its fractions were preserved in a desiccator for future use.

2.5 Antimicrobial Assay of methanol Extract of *Cola lepidota* K. Schum Seeds

(i) Collection of microbial test organisms
Isolates were obtained from the Microbiology Laboratory of the University Health Centre, Akwa Ibom state University, Mkpato Enin, Nigeria. The organisms included: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. These organisms were sub-cultured and preserved as pure cultures on Nutrient agar slants and stored at low temperatures until required.

(ii) Determination of extract concentrations
The extracts were dissolved using sterile water to constitute different concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5mg/mL.

(iii) Determination of antimicrobial assay of extract on selected test organisms:

Antibacterial activity of the extracts was evaluated using the well in agar diffusion technique (Okeke, 2001). The diluted test organisms on Nutrient broth for bacterial isolates were further sub cultured to Peptone water and cells adjusted to McFarland Turbidity standard. 0.5 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 inoculum on the surface of the medium and was allowed 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 impregnated with the test organisms. In each of the wells previously impregnated with the test organisms, 100 mg of the extract dilution of different concentrations were introduced into the wells. Control experiments were set up alongside with the extracts using commercial antibiotics drugs (Ciprofloxacin and



Gentamycin). All plates were left on the bench for 1 hour before incubating at 37°C for 24 hours for bacterial isolates. After incubation, the antimicrobial assay was determined by measuring the diameter of the zone of inhibition diameter (ZID) observed (MacFarland standard) (Ulusoylu *et al.*, 2001; Ekong, 2004). Each test was performed in triplicates and the average of the results was attained.

(iV) Determination of Minimum Inhibitory Concentration (MIC)

The MIC of *Cola lepidota* K. Schum extract was determined in triplicates using the agar plate's method as described by the Clinical and Laboratory Standards Institute (2011). To obtain this, 0.25 mL from the last concentration of the extract that had activity for an organism were further diluted. 0.25 mL from each diluted extract was introduced into sterile plates. Nutrient agar (NA) was poured into those plates, swirled and left on the bench to set. These were used for bacterial isolates. 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 bacterial growth. The minimum concentrations that inhibit the growth of any test organisms were noted as MIC for the extract on the test organism (Ekong, 2004).

3.8 Antibiotic Susceptibility Testing

The antibiotics susceptibility tests were carried out using the Mueller-Hinton agar. The Kirby-bauer disc diffusion method was used to determine the susceptibility of the isolates

(*Staphylococcus aureus*, *B. subtilis*, *E. coli* and *Pseudomonas sp.*) to the following antibiotics: Ciprofloxacin and Gentamycin. The standard suspension of each isolate that matched 0.5 McFarland standard were used to swab the surface of Mueller Hinton agar plate of which the Kirby-bauer disc containing antibiotics impregnated discs were aseptically placed on the plates. The plates were allowed to dry for 15mins and were incubated overnight at 37°C. The results of the susceptibility testing were interpreted based on the guidelines of the Clinical Laboratory Standard Institute. Diameter of zone of inhibition around each antibiotics disc will be recorded in millimeter (CLSI, 2020).

3.9 Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean \pm standard deviation (SD). All data collected were used exclusively for research purposes and stored securely in accordance with data protection protocols.

3.0 Results and Discussion

3.1 Results

3.1.1 Phytochemical Screening

The phytochemical screening from Table 1 and Table 2 revealed the presence of saponins, alkaloids, tannins, flavonoids, cardiac glycosides and the absence of anthraquinones in the fruit epicarp extract of *Cola lepidota* K. Schum while saponins, alkaloids, flavonoids, cardiac glycosides were present in the seed extract but the absence of tannin and anthraquinones.

Table 1: Results of phytochemical Screening of fruit of *Cola lepidota* K. Schum

Phytochemical	Test	Observation	Inference
Alkaloids	Dragendorff's reagent	A red precipitate	+
Tannins	Ferric chloride	Blue black precipitate	+
Cardiac glycosides	Salkowski's test	Brown ring formed at interphase	+



	Keller Kiliani test	Brown ring formed at interphase	+
	Lieberman's test	A pink to blue to brown colouration at the interphase	+
Flavonoids	Magnesium metal test	Orange colouration	+
	Sodium hydroxide test	A yellow colouration	+
	Ammonia test	A yellow colouration	+
Anthraquinones	Modified Borntrager's test	No red, violet or pink colouration	—
	Borntrager's test	No red, violet or pink colouration	—
Saponins	Frothing test	Persistent frothing	+

Keys: + present; - absent

Table 2: Results of phytochemical Screening of Seed extract of *Cola lepidota* K. Schum

Phytochemical	Test	Observation	Inference
Alkaloids	Dragendorff's reagent	A red precipitate	+
Tannins	Ferric chloride	No Blue black precipitate	—
Cardiac glycosides	Salkowski's test	Brown ring formed at interphase	+
	Keller Kiliani test	Brown ring formed at interphase	+
	Lieberman's test	A pink to blue to brown colouration at the interphase	+
Flavonoids	Magnesium metal test	Orange colouration	+
	Sodium hydroxide test	A yellow colouration	+
	Ammonia test	A yellow colouration	+
Anthraquinones	Modified Borntrager's test	No red, violet or pink colouration	—
	Borntrager's test	No red, violet or pink colouration	—
Saponins	Frothing test	Persistent frothing	+

Keys: + present; - absent



3.1.2 Antibacterial activity against all tested bacterial strains

Table 3 revealed that the seed extract of *Cola lepidota* K. Schum exhibits antimicrobial activity against all tested bacterial strains. It was observed that zone of inhibition increases with extract concentration though *Staphylococcus aureus* was the most susceptible strain with zone of inhibition of 30mm at 100mg/ml. No zone inhibition was observed at 12.5mg/ml in *B. subtilis*, *E. coli* and *Pseudomonas sp*

Table 4 revealed that the epicarp extract of *Cola lepidota* K. Schum exhibits antimicrobial activity against all tested bacterial strains. It

was observed that zone of inhibition also increases with increasing extract concentration. *B. subtilis* was slightly more susceptible at higher concentrations (100mg/ml and 50mg/ml). But at lower concentrations (12.5mg/ml), *Pseudomonas sp.* shows no zone of inhibition (NZ).

In Table.5 both seed and epicarp extracts show antimicrobial activity against the tested bacteria, but the seed extract was more potent against *E. coli*. though *E. coli* was highly sensitive to the seed extract, with an MIC of 0.25mg/mL.

Table 3: Seed Extract Mean Zone of Inhibition

Samples(mg/ml)	<i>Staphylococcus aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>E. coli</i> (mm)	<i>Pseudomonas sp.</i> (mm)
100	30.0	20.0	20.0	20.0
50	20.0	15.0	15.0	18.0
25	18.0	12.0	12.0	15.0
12.5	15.0	NZ	NZ	10.0

*Data represents the mean of triplicate determinations:

Table 4: Fruit Epicarp Mean Zone of Inhibition

Samples(mg/ml)	<i>Staphylococcus aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>E. coli</i> (mm)	<i>Pseudomonas sp.</i> (mm)
100	20.0	22.0	20.0	20.0
50	18.0	20.0	12.0	15.0
25	15.0	15.0	12.0	10.0
12.5	16.0	12.0	10.0	NZ

*Data represents the mean of triplicate determinations:

Table 5: Bacterial Minimum Inhibition Concentration

Samples(mg/ml)	<i>Staphylococcus aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>E. coli</i> (mm)	<i>Pseudomonas sp.</i> (mm)
Seed Extract	12.5	12.5	0.25	6.25
Epicarp Extract	12.5	12.5	12.5	6.25

*Data represents the mean of triplicate determinations:

3.1.3 Comparative analysis with different antibiotics

In Table 6, Ciprofloxacin exhibits significant antimicrobial activity against all tested bacterial strains (*Staphylococcus aureus*, *B. subtilis*, *E. coli*, and *Pseudomonas sp.*). The



ZOI decreases with decreasing Ciprofloxacin concentration (from 50mg/ml to 25mg/ml).

In Table 7, at 8mg/mL, Gentamycin shows strong antimicrobial activity against all four bacteria, with a zone of inhibition of 50.0mm for each.

At 4mg/mL, the activity decreases for *Staphylococcus aureus*, *B. subtilis*, and *E. coli*, with a zone of inhibition of 30.0mm for each. However, the activity against *Pseudomonas sp.* remains the same, with a zone of inhibition of 50.0 mm.

Table 3.6: Comparative analysis with different antibiotics (Ciprofloxacin)

Ciprofloxacin(mg/ml)	<i>Staphylococcus aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>E. coli</i> (mm)	<i>Pseudomonas sp.</i> (mm)
50mg	30.0	30.0	25.0	25.0
25mg	20.0	20.0	20.0	20.0

*Data represents the mean of triplicate determinations:

Table 3.7: Comparative analysis with different antibiotics (Gentamycin)

Gentamycin (mg/ml)	<i>Staphylococcus aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>E. coli</i> (mm)	<i>Pseudomonas sp.</i> (mm)
8mg	50.0	50.0	50.0	50.0
4mg	30.0	30.0	30.0	50.0

*Data represents the mean of triplicate determinations

3.1.4 Bacterial Minimum Inhibition Concentration

In Table 8, the fruit epicarp extract did not inhibit or kill the bacteria at the tested concentrations suggesting less antibacterial potential.

Table 8: Minimum Bacterial Concentration of the Fruit Epicarp Samples

Epicarp (mg/ml)	Samples	<i>Staphylococcus aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>E. coli</i> (mm)	<i>Pseudomonas sp.</i> (mm)
25		+	+	+	+
12.5		+	+	+	+
6.25		+	+	+	+
3.125		+	+	+	+

*Data represents the mean of triplicate determinations:

3.2 Discussion

Antimicrobial resistance has been increased seriously in recent days. With the increasing prevalence of antimicrobial resistance, there is a growing need for alternative treatments that are effective, safe, and sustainable (O'Neill, 2016). This led the scientists to focus on discovering the antimicrobial activity of different plants and herbs that has the potential to contribute to the development of new

antimicrobial agents from natural sources (Boucher *et al.*, 2013).

Bioactive compounds are specific types of phytochemicals that have been shown to have biological activity, meaning they can interact with living organisms and produce a response. These compounds can have a range of effects on human health, from antioxidant and anti-inflammatory properties to antimicrobial and anticancer activities (Iwu, 2014).



The Phytochemical screening carried out on the methanol seed extract revealed the presence of saponins, tannins, alkaloids, cardiac glycosides and Flavonoids while the fruit epicarp extract revealed the presence of saponins, alkaloids, cardiac glycosides, Flavonoids. Tannin was absent in the fruit epicarp. Anthraquinones was not present in both extracts. These phytochemical compounds are known to play important roles in bioactivity of medicinal plants and significant impact on the overall and wellbeing (Benmehdi *et al.*, 2012; Ekpo *et al.*, 2012; Imieje, *et al.*, 2013)

Flavonoids and tannins are also known to exhibit a wide range of biological activity partly due to their antioxidant effect (Guyen, 2019; Uffia *et al.*, 2024). It is also important to note that there was no free anthraquinones present in the extract. Result of the analysis is in cohesion with works of Benmehdi *et al.*, (2012) and Vincent *et al.*, (2013)

The study was in line with the works Ogidi *et al.*, (2020) and Uwanta *et al.*, (2020), who evaluated the phytochemical composition and antioxidant activity of *Cola lepidota* K. Schum seed extracts whose results showed that the extracts contained various phytochemicals, including phenols, flavonoids, tannins, saponins, and alkaloids.

The results of this study demonstrate the antibacterial potential of *Cola lepidota* K. Schum seed and fruit epicarp extracts against various bacterial strains. The extracts exhibited varying degrees of inhibition against the test microorganisms, with the seed extract showing more potent activity against *E. coli* (MIC of 0.25mg/ml) compared to the fruit epicarp extract.

The antibacterial activity of the seed extract against *Staphylococcus aureus*, *B. subtilis*, *E. coli*, and *Pseudomonas sp.* is notable, with zone of inhibition values of 30.0mm, 20.0mm, 20.0mm, and 20.0mm respectively, at a concentration of 100mg/ml. The fruit epicarp extract also showed antibacterial activity, with zone of inhibition values of 20.0mm, 22.0mm,

20.0mm, and 20.0mm, respectively, at a concentration of 100mg/ml. These findings are consistent with previous studies on other plant extracts, which have reported varying levels of antimicrobial activity (Owolabi, *et al.*, 2011; Odeyemi, 2020). Inhibiting protein production and microbial growth and ability to interact with microbial cell membranes, disrupting their integrity and leading to the leakage of essential cellular components could be as a result of presence of some bioactive compounds in *Cola lepidota* K. Schum extracts which interfere with amino acid synthesis (Wang *et al.*, 2017; Solomon *et al.*, 2019)

The comparative analysis with Ciprofloxacin and Gentamycin revealed that the extracts have antibacterial activity comparable to these standard antibiotics, although the antibiotics showed more potent activity at higher concentrations. For example, Ciprofloxacin showed zone of inhibition values of 30.0mm, 30.0mm, 25.0mm, and 25.0mm against *Staphylococcus aureus*, *B. subtilis*, *E. coli*, and *Pseudomonas sp.*, respectively, at a concentration of 50mg/mL. Gentamycin showed zone of inhibition values of 50.0mm against all four bacteria at a concentration of 8mg/mL.

The MIC values for the seed and fruit epicarp extracts varied depending on the microorganism and extract concentration. The seed extract showed more potent activity against *E. coli*, with an MIC of 0.25mg/mL, while the fruit epicarp extract showed MIC values of 12.5mg/mL against *Staphylococcus aureus* and *B. subtilis*. These findings are consistent with the results of Eloff (1998) and Solomon *et al.*, (2019), who reported similar MIC values for plant extracts against various microorganisms.

The study also confirmed the assertion of Oranusi *et al.*, (2019) who evaluated the antimicrobial activity of ethanolic extracts of *Cola lepidota* fruits against *B. subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The results showed that the



extracts did not exhibit antimicrobial activity against the test microorganisms suggesting seeds and epicarp extract to exhibit more potent antibacterial activities than the fruit extract.

4.0 Conclusion

This study revealed that *Cola lepidota* possesses high phytochemical potential that supports its traditional use in managing degenerative diseases. The seed and fruit epicarp extracts demonstrated antibacterial activity against the tested bacterial strains, with the seed extract showing the highest potency against *Escherichia coli* at a minimum inhibitory concentration of 0.25 mg/mL, while the fruit epicarp extract exhibited moderate antibacterial activity. These results indicate that the bioactive constituents of *Cola lepidota* may serve as promising antimicrobial agents, contributing scientific evidence for its ethnomedicinal application. In conclusion, the findings establish the therapeutic potential of the plant extracts and justify further investigation. It is recommended that future studies isolate and characterize the active compounds, evaluate their mechanisms of action, and assess possible synergistic effects with conventional antibiotics for the development of new treatments for infectious diseases.

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- Competing interests**
- There are no known financial competing interests to disclose
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- All authors contributed to this work. Ifiok Dominic Uffia designed and supervised the study and revised the manuscript. Ofonimeh Emmanuel Udofia performed laboratory experiments, analyzed data, and drafted the paper. Iniobong Bruno Nsien authenticated samples and assisted data interpretation. Rose Okopide Esen supported phytochemical analysis and review, while Christiana Samuel Udofia assisted antibacterial testing, literature review, and proofreading. All authors approved the final manuscript.

