Nutritional and Bioactive Properties of *Cola Lepidota &Cola Pachycarpa (Ochicha)* Seed Extracts on Three Strains of Organisms

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DOI: https://doi.org/10.51584/IJRIAS.2024.908059

Received: 15 August 2024; Revised: 25 August 2024; Accepted: 29 August 2024; Published: 18 September 2024

ABSTRACT

This study evaluated the phytochemical composition, antioxidant activity, and antimicrobial potentials of *Cola lepidota* and *Cola pachycarpa* seeds. Ethanol and distilled water were used as solvents for phytochemical extraction, while the test bacterial strains were obtained from the National Veterinary Research Institute, Vom, Jos, Plateau State. The plant bioactive compounds were detected using qualitative and quantitative phytochemical evaluation, including gas chromatography-mass spectrometry (GC/MS) analytical methods. Proximate composition was determined, and antioxidant activity was assessed using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH), reducing power activity, and ABTS+ assays. The antibacterial activity of the seed extract was evaluated via the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) using the agar-well diffusion method. The result of quantitative analysis showed lower concentrations of phenols (1.26 mg/g and 1.35 mg/g), flavonoids (0.87 mg/g and 0.93 mg/g), tannins (1.08 mg/g and 1.17 mg/g), saponins (1.49 mg/g and 1.57 mg/g), and alkaloids (0.43 mg/g and 0.46 mg/g) in *C. lepidota* than in *C. pachycarpa.* Conversely, the mineral composition was higher in *C. lepidota*, recording 3.92%, 1.02%, 0.17%, 0.02%, and 0.01% for potassium, phosphorus, calcium, sodium, and iron, respectively. The extracts showed no inhibitory activity against the bacterial strains: *Escherichia coli* ATCC 0157, *Staphylococcus aureus* ATCC 6538, and *Bacillus substilis ATCC* 6633. As such, failed to justify the ethno-pharmaceutical uses in the treatment of bacterial diseases. However, they demonstrated rich innate antioxidant activity and may be helpful in the prevention or reduction of oxidation or similar associated disorders in humans.

Keywords: ethno-pharmaceutical, antioxidant, antibacterial activity.

INTRODUCTION

An increasing number of studies are being conducted to explore natural compounds rich in antioxidants and antimicrobial properties because of their significance in treating various chronic disorders, such as cancer and cardiovascular disease. Approximately two-thirds of drugs approved worldwide are predicted to be plant derivatives (Patridge *et al.,* 2016). Formulations from plants have gained popularity and importance in recent years because of their efficacy, safety, and cost effectiveness. They have been used as antibacterial, antioxidant, antiulcer, anti-inflammatory, antiviral, immune-stimulants and anticancer agents especially in developing countries where infectious diseases are endemic and health services and hygiene facilities are inadequate.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

Estimations made by the World Health Organization (WHO) revealed that 80% of people who live in developed countries generally use traditional medicine (Rahim and Khan, 2006). Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (Hashem *et al*., 2010), the use of medicinal plants is very wide spread in many parts of the world because it is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects. It has been proven that plants and other natural products are template for the development of new scaffolds of drugs (Newman and Cragg, 2007). Africa is blessed with enormous biodiversity of natural product for healing practices (Hashem *et al*., 2010).

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds and the medicinal values of these plants lie in their phytochemical elements, which produce definite and diverse physiological and pharmacological response in the human body (Lai and Roy, 2004).

According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals (‘phyto- ‘from Greek –*phyto* meaning ‘plant’) or phyto-constituents and are responsible for protecting the plant against microbial infections or infestations by pests (Liu, 2004; Nweze *et al*., 2004).

The control of infectious diseases is badly endangered by the rise in the number of microorganism that are resistant to antimicrobial agents. This is because infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death (Mouhssin et al, 2017). Fruit trees in Africa constitute one of the best tools for preventing diseases caused by lack or insufficient supply of vitamins in diet. Fruit are good source of protein, fat, carbohydrate and minerals. Nutritionally, they are believed to contain beta carotene which acts as antioxidant (Bello *et al.,* 2008). Recent findings have revealed that fruits contain bioactive compounds such as polyphenols, alkaloids, saponins and anthraquinones etc, which have some medicinal potential (Mohammed *et al.,* 2010).

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). *C. lepidota* belongs to the group of Monkey kola, a member of the family *Malvaceae* and subfamily *Sterculiaceae*. The Monkey kola varieties include red (*C. lateritia*), yellow (*C. lepidota*) and white (*C. pachycarpa*) types (Okudu and Ene-Obong, 2015). About forty Cola species have been described in West Africa, however, in Nigeria only about twenty-three species are known and some are used in traditional medicine as a stimulant, to prevent dysentery, headache and to suppress sleep (Essien *et al.,* 2015). 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 *et al.,* 2015).

Plants are the richest sources of bioactive compounds and have been the basis of many traditional medicines throughout the world for thousands of years (Ingale and Hivrale, 2010). Natural products, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos *et al.,* 2006). Clardy and Walsh (2004) reported that small molecules from medicinal plants called natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases. The medicinal value of the plants useful for healing and cure of human diseases is attributed to presence of phytochemical constituents (Nostro *et al.,* 2000). Among these phytochemicals are flavonoids, alkaloids, phenols, saponins etc (Edeoga *et al.,* 2005). According to Ogbu *et al*. (2007), tropical African sub-regions are home to many useful medicinal plants, however, there are many of them whose potentials are yet to be harnessed or domesticated in tropical African Sub-regions.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

MATERIALS AND METHODS

This study was carried out using random sampling method and was divided into five experimental phases.

Sample collection

The two medicinal plants, *Cola lepidota* (yellow species) and *Cola pachycarpa* (white) were collected in local markets which include Ahiaohuru market, Arochukwu bush market and Upgate all in Abia state Nigeria, purchased from at least three randomly selected vendors in the various markets.

Identification of samples

Freshly collected fruit samples were identified and authenticated by a Taxonomist in the Department of Plant Science and Biotechnology Abia State University Uturu.

Extraction Procedure

Ethanol and water were used as the solvent for the seed extract. 700g of each of the powdered seeds (*Cola lepidota* and *Cola pachycarpa* seeds) was soaked in 1000ml (70% w/v ethanol) of absolute ethanol and (stirred at every 3h) left for 24 hours at room temperature. The filtrates were evaporated to dryness using hot air oven set at 70^0C.The extracts were filtered using No.1Whatman filter paper into a clean beaker. The obtained extracts were weighed and stored until use. (Burdass *et al*., 2006). 700g of each of the powdered plant seeds (*Cola lepidota* and *Cola pachycarpa* seeds) was soaked in 1000ml distilled water and (stirred every 3h) for 12 hours at room temperature. The filtrates were evaporated to dryness using water bathe set at 70^0C. The obtained extracts were weighed and stored until use.

Test organisms

The bacterial strains used were *E. coli* 0157:H7, *S. aureus* ATCC 6538 and *B. substilis* ATCC 6633 obtained from National Veterinary Research Institute, Vom, Jos, Plateau State. The strains were reactivated in normal saline and sub-cultured at 37°C on Nutrient media.

Preparation of stock solution of extract

Each plant (*Cola lepidota* and *Cola pachycarpa* seed extract) extract was prepared by dissolving 5g of plant extract in10ml of normal saline to yield 500mg/ml. Further dilution was made by transferring from the stock solution and double dilution was done to yield lower concentrations of 250, 125, 62.5, and 31.25mg/ml.

Phytochemical Screening

Qualitative Screening

Chemical tests were carried out on the powdered specimens using standard procedures to identify the constituents as described by Edeoga *et al.,* 2005

Test for Tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Saponin

Exactly 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

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Test for Tepernoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of tepernoids.

Test for Alkaloids

Amount of 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. A milliliter of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

Test for Flavonoids

Exactly 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange coloration was indicative of the flavonoids.

Quantitative Determination of the chemical constituents

Alkaloid Determination by Method of Harborne (1973)

Amount of 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter (1/4) of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin determination by Method of Van (1981)

Five hundred milligram (500 mg) of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm in a spectrophotometer within 10 min.

Determination of saponins

This was done by the double solvent extraction gravimetric method (Harborne, 1973). Five grams (5g) of the sample was mixed with 50ml of 20% aqueous ethanol solution and incubated for 12 hours at a temperature of 55^{o}C with constant agitation. After that, the mixture was filtered through Whatman No 42 grades of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 minutes and the extracts weighed together. The combined extract was reduced to about 40ml by evaporation and then transferred to a separating funnel and equal volume (40ml) of diethyl ether was added to it. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. This aqueous layer was re-extracted with the ether after which its pH was reduced to 5 with drop-wise addition of dilute NaOH solution. Saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of normal butanol. The combine extract (ppt) was washed with 5% NaCl solution and evaporated to dryness in a previously weighted evaporating dish. The saponin was then dried in the oven at 60^{o}C (to remove any residual solvent) cooled in a desiccator and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

Flavonoid Determination by Method of Boham and Kocipai (1994)

Ten grams (10g) of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmans filter paper No 42 (125 mm). The filtrate was

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later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Determination of Proximate Composition

Moisture Content Determination

Moisture content was determined using the method described by Association of Official Analytical Chemist (AOAC, 2005). Five (5.0g) of each sample was weighed into Petri dish of known weight and then dried in the oven at 105±1^{o}C for 4hrs. The samples were cooled in a desiccator and weighed. The moisture content was calculated as follows:

Percentage moisture content = (*change in weight / initial weight of sample before drying*) x (100/1)

Fat Content determination

Fat content was determined using the method of (AOAC, 2005). Five grams of sample was weighed using a chemical balance and wrapped in a filter paper. It was then placed in an extraction thimble cleaned and dried in an oven, and cooled in desiccators before weighing. Then, about 25ml of petroleum ether solvent was measured into the flask and the fat extracted. After extraction, the solvent was evaporated by drying in the oven. The flask and its contents were cooled in desiccators and weighed. The percentage fat content was calculated as follows:

(*weight of the extract / weight of the sample*) × 100/1

Crude Protein Determination

The protein was determined using a micro-kjeldahl method as described by (AOAC, 2005) which involve wet digestion, distillation and titration. The protein content was determined by weighing about 0.5g of sample into a boiling tube that contained 25ml concentrated H_2SO_4 and one catalyst tablet containing 5g H_2SO_4, 0.15g TiO_2. Tubes were heated at low temperature for digestion to occur. The digest was diluted with 100ml distilled water, 10ml of 40% NaOH, and 5ml $Na_2S_2O_3$, anti-bumping agent was added, and then the sample was diluted with 10ml of Boric acid. The NH_4 content in the distillate will be determined by titrating with 0.1N standard HCl using a 254ml burette. A blank was prepared without the sample. The protein value obtained was obtained by a conversion factor, and the result expressed as the amount of crude protein.

% crude protein = (((b-a) × 0.1 ×14.00) /W) X 100 X (6.25/1000)

Where W = weight in grams of sample analysed

a = volume (ml) of 0.1N of H_2SO_4 used in blank titration

b = volume (ml) of 0.1N of H_2SO_4 used in simple titration

14.00 = Atomic weight of nitrogen

6.25 = the protein-nitrogen conversion factor

1000 = the conversion of mg N/100 g to g N/100 g sample.

Total Ash Determination

Ash content was determined using the method of (AOAC, 2005). About 5g of each sample was weighed into crucibles in duplicate, and then the samples were incinerated in a muffle furnace at 550 ^{o}C until a light grey ash was observed and a constant weight obtained. The samples were cooled in desiccators to avoid absorption of moisture and weighed to obtain ash content. Percentage ash was calculated using the formula:

$((W_2 - W_1)/W) \times (100/1)$

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Where W= Dry weight of food sample,

W_1= weight of crucible,

W_2 = weight of crucible and ash

Carbohydrate Determination

The carbohydrate content was calculated by difference as the Nitrogen Free Extractive (NFE), a method separately described by James (2012). The nitrogen free extractive will be calculated as

% NFE = 100-% (a +b +c +d +e)

Where; a = protein

b = fat

c = fibre

d = ash

e = moisture.

Mineral Element Composition

The elemental composition of the samples was determined following the method of Idouraine *et al* (1996). The sample (1 g) was dried-ashed in a muffle furnace at 550°C for 5 h until a white ash was obtained. The minerals were extracted from ash by adding 3 ml of concentrated HNO_3 (63%). The digest was carefully filtered into 100 mL standard bottle and made up to mark with distilled water. Minerals elements (Na, K, Ca, Mg, Zn, Cu, Mn and Fe) were estimated with the use of a flame photometer (Jenway model PFP7) and atomic absorption spectrophotometer (Perkin Elmer model 703, USA). The instruments were calibrated with standard solutions containing known amounts of the minerals being determined, using analytical reagents.

Antioxidant Composition

Determination of antioxidant activity of crude extracts of *C. lepidota* seed. The method of Ruch *et al*. (1989) was used, by following the description (Oranusi *et al*. 2013). Different concentrations (25.000; 12.500; 6.250 and 3.125 mg mL–1) of the extracts were prepared. To each extract (100) was added H_2O_2 (600 µL) in 0.1 M phosphate buffer (pH 7.4). Samples were incubated at room temperature in the dark for 10 minutes. A negative control was set up in parallel with the entire reagent except extract or standard. Absorbance was read at 230 nm against a blank solution containing the phosphate buffer without H_2O_2, using the spectrophotometer model M106 (Spectronic Campsec, UK). The percentage of H_2O_2 scavenging of the extracts and standard compounds was calculated. Total flavonoid content assay to distilled water (490 µL), the extract was added (10 µL) and followed by 5% sodium nitrate (30 µL) and 10% aluminium chloride (30 µL). Samples were incubated at room temperature for 5 min and 1 M NaOH (200 µL) was added, followed by distilled water (240 µL), and vortexed thoroughly. Absorbance was read at 570 nm, using the Genesys, G105 UV-VIS spectrophotometer (Thermo Fisher Scientific, USA). Standard pyrocatechol was used as the control, mean of the triplicate analysis was recorded and results were expressed as milligrams of catechin equivalents per 100 g of sample (Oranusi et al. 2013). Total phenol content assay. The Folin-Ciocalteu method was used as was described by Alothman et al. (2009). To the extracts (10 µL), distilled water (600 µL) was added and followed by the 10% Folin-Ciocalteu reagent (50 µL). Then 7% Na_2Co_3 was added (150 µL) and vortexed thoroughly for 2 minutes. It was incubated for 8 minutes at room temperature and then distilled water was added (190 µL). Afterwards, it was allowed to stand for 2 h and absorbance was read at 765 nm, using the Genesys, G105 UV-VIS spectrophotometer (Thermo Fisher Scientific, USA). Gallic acid was used as the standard, mean of the triplicate analysis was recorded and results were expressed as milligrams of gallic acid equivalent per 100 g of sample.

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Gas chromatography-Mass spectrometry analysis

The Gas chromatography-Mass spectrometry (GC-MS) analysis of the extract was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter, and 0.25mm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51ml/min. injector and mass transfer line temperature were set at 200 and 240^{o}C respectively. The oven temperature was programmed from 70 to 220^{o}C at 10^{o}C/min, held isothermal for 1min and finally raised to 300^{o}C. At 10^{o}C/min. 2ml of water solution of sample was manually injected in the split less mode, with a split ratio of 1:40 and with mass scan of 50-600 amu. Total running time of GC-MS is 35min. The relative percentage of extract constituent was expressed as a percentage with peak area normalization. Interpretation of mass spectrum of extract was conducted using the database of National Institute of Standard and Technology (NIST) library having more than 62,000 spectral patterns. The spectrum of the compounds was compared with the spectrum of National Institute of Standard and Technology (NIST) library database.

Antimicrobial Assay

Agar well diffusion technique was used to determine the antibacterial activity of the extracts. Agar medium was prepared following manufacturer's instructions and autoclaved (121°C for 15 min). The agar was allowed to cool to 40^{0}c and 20ml of the agar was aseptically dispensed into each plates. The Mueller Hinton agar was allowed to solidify. Suspensions of the first and second organism were made in a McCarthy bottle and the turbidity was matched with that of 0.5 McFarland standards. The inoculum was swabbed on to the plate using sterile swab stick. A standard sterile cork-borer of 8 mm diameter was used to cut uniform wells on the surface of the agar plate. A volume of 50µl of each of the extracts containing different concentrations was loaded in each well (8 mm diameter). The plates were incubated for 24 h at 37°C and the zone of inhibition was measured. Normal saline was used as the negative control for each extracts (Balouiri et al., 2016).

Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration or the highest dilution of an antimicrobial agent that prevents visible growth after 18-24 hours of incubation. It was determined by making dilution of various concentrations (100, 50, 25, 12.5, 6.25, 3.12 and 1.56mg/ml) of the ethanol extract in test tubes. 1ml stock solution of the plant extract 100mg/ml was diluted in sterile test tube containing 0.95ml of Mueller Hinton Broth (MHB) to obtain further dilution. Serial dilution techniques were employed by transferring 1ml from the first test tube to the second test tube and from the second to the third. This was continued to the seventh test tube from where 1ml was discarded to give concentration of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56mg/ml. Another test tube was also prepared with MHB (control) of the test tube which inoculated with standard suspension (50µl) of the test organism and incubated at 37°C overnight. After incubation, the turbidity in each tube was checked. The tube that contains the lowest concentration which showed no turbidity i.e. a clear view, was observed to be the MIC of the antimicrobial agent for the organism tested, the lower the MIC, the more susceptible is the test organism.

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antimicrobial agent required to kill a microorganism. This was determined by streaking the content of the tubes used for MIC determination which were showing reduced turbidity on freshly prepared nutrient agar plates. The MBC was then identified as the concentration that completely inhibited the growth of the test bacteria.

RESULTS

Table 1. Qualitative Phytochemical Composition of Samples

Samples I. D	Total Phenol (mg/g)	Total Flavonoids (mg/g)	Total Tannins (mg/g)	Total Saponins (mg/g)	Total Alkaloids (mg/g)
Cola Lepidota	+	+	+	+	+
Cola Pachycarpa	+	+	+	+	+

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Table 2. Quantitative Phytochemical Composition of Samples

Samples I. D	Total Phenol (mg/g)	Total Flavonoids (mg/g)	Total Tannins (mg/g)	Total Saponins (mg/g)	Total Alkaloids (mg/g)
Cola Lepidota	1.26±0.01	0.87±0.02	1.08±0.02	1.49±0.01	0.43±0.01
Cola Pachycarpa	1.35±0.03***	0.93±0.02*	1.17±0.03***	1.57±0.03**	0.46±0.01

Values are presented as mean ± SD with n = 3. *significantly different ($p<0.05$), ** significantly different ($p<0.01$), *** significantly different ($p<0.001$)

Table. 3 Proximate Analysis of Samples

Sample ID	Moist content (%)	Ash (%)	Fat (%)	Protein (%)
Cola Lepidota	4.076±0.02****	7.077±0.02	1.207±0.03	25.632±0.03****
Cola Pachycarpa	3.700±0.02	8.660±0.02****	1.400±0.02****	23.357±0.02

Values are presented as mean ± SD with n = 3. **** Significantly different ($p<0.0001$)

Table. 4 Minerals Composition of Samples

Sample ID	P (%)	Ca (%)	Mg (%)	K (%)	Na (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
Cola Lepidota	1.05± 0.03	0.18 ±0.03	0.23 ±0.03	3.83 ±0.13	79.53 ±0.03	13.85 ±0.02	151.15± 0.02	5.02± 0.02	82.07±0. 02
Cola Pachycarpa	0.94± 0.05*	0.17 ±0.02	0.25 ±0.02	3.87 ±0.02	89.82± 0.02****	17.94± 0.06****	186.03± 0.01****	7.59± 0.02****	92.0± 0.02****

Values are presented as mean ± SD with n = 3. *significantly different ($p<0.05$), **** significantly different ($p<0.0001$)

Table. 5 Antioxidant Activities of Samples

Sample ID	DPPH* SC_{50} (mg/ml)	ABTS*+scavenging ability (mmol TEAC/g)	Reducing power (mg GAE/g)
Cola Lepidota	12.8±0.09**	82.2±0.58	1.59±0.02
Cola Pachycarpa	11.9±0.09	87.5±0.34****	1.73±0.02

Values are presented as mean ± SD with n = 3. **significantly different ($p<0.01$), **** significantly different ($p<0.0001$)

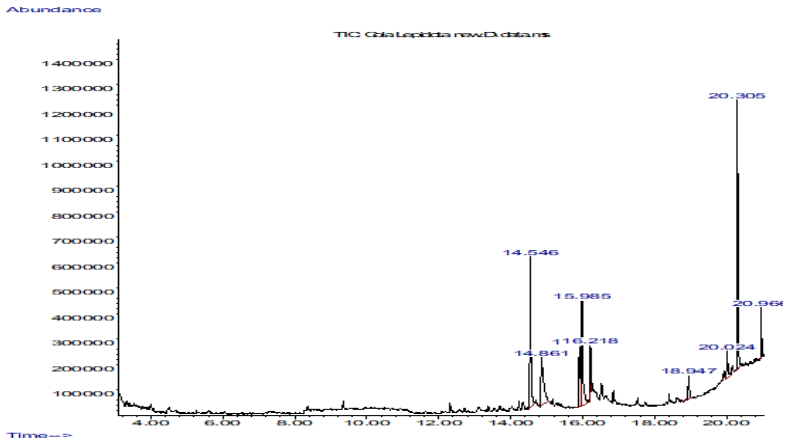

Figure 1. GC-MS Chromatogram

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Table 6. Antimicrobial Activity of *Cola lepidota* Seeds Extracts on Organisms

Diluent in mg/ml	Different Conc.	Microorganisms Type strain	Zone of inhibition 18-24hr
Water extract mg/ml	Undiluted	*E. coli* 0157:H7	R
	250mg/ml	*S. aureus* ATTC 6538	R
	125mg/ml	*B. substilis* ATCC 6633	R
	62.5mg/ml		R
	31.25mg/ml		R
Ethanol extract mg/ml	Undiluted		R
	250 mg/ml		R
	125 mg/ml		R
	62.5mg/ml		R
	31.25mg/m		R

Table 7. Antimicrobial Activity of *Cola pachycarpa* Seeds Extracts on Organisms

Diluent in mg/ml	Different Conc.	Microorganisms Type strain	Zone of inhibition 18-24hr
Water extract mg/ml	Undiluted	*E. coli* 0157:H7	R
	250mg/ml	*Staph. aureus* ATTC 6538	R
	125mg/ml	B. substilis ATCC 6633	R
	62.5mg/ml		R
	31.25mg/ml		R
Ethanol extract mg/ml	Undiluted		R
	250 mg/ml		R
	125 mg/ml		R
	62.5mg/ml		R
	31.25mg/m		R

R- resistant

DISCUSSION

The fruit part is cherished as food which is an added advantage due to the direct relationship of sodium intake with hypertension in human (Soetan *et al.*, 2010). The zinc content could mean that the seeds can play a valuable role in the management of diabetes, which results from insulin malfunctioning. Zinc is significant for the production of insulin in the body (Okwu, 2004). Eneobong *et al*. (2016) also indicated that calcium and magnesium were the most abundant minerals in the fruit pulp of *C. pachycarpa* and *C. lepidota*. High amount of some essential minerals in the endocarp of *C. lepidota* relative to the exocarp has been documented by Osabor *et al*. (2015).

Potassium was the most abundant mineral in the samples recording (3.92% and 3.87%) for *C. lepidota* and *C. pachycarpa* respectively. High values could be due to high uptakes of potassium by the plant from soil. The values are higher compared to (59.5mg/100g) reported for a related species (*C. citratus*) in another study by Emmanuel and Oludele (2010) and (24mg/100g) *C. spicata* (Suleiman *et al.*, 2011). And lower compared to (1082mg/100g) *C. citrates*. This suggests that the two species could be good source of potassium. Potassium is essential in the maintenance of cellular water balance, pH regulation in the body and protein as well as carbohydrate metabolism. This study further presented *C. lepidota* as a rich source of minerals, specifically potassium, (K), Phosphorus (P) and a moderate amount of magnesium and calcium. Thus, it may be safe to mention that these wild fruits may be included in the diet as a complementary source of these minerals.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

The sodium content of *C. lepidota* and *C. pachycarpa* are (15.83mg/100g) and (13.17mg/100g) respectively. The values are low when compared to (54.8mg/100g) *C. citrates* (Emmanuel and Oludele 2010). And higher when compared to (1.06mg/100g) *C. citrates* and (7.2mg/100g) *M. spicata* (Suleiman *et al.*, 2011). The low sodium content is desirable because high dietary sodium has been associated with hypertension.

Ogbu *et al*. (2007) showed that *C. lepidota* contain moisture (82.6 g/100g). The moisture content of *C. lepidota* is relatively lower when compared with other varieties of the fruit as was described by Kwazo and Aletan (2019). The low moisture content observed in the samples contradicts earlier results of Osabor *et al.* (2015) where they reported a higher moisture content of 22.00 + 0.12%. Low moisture content of the seeds could be an advantage as it helps to prevent microbial attacks and allows for long storage capacity. The values are comparable with those of *C. citratus* (3.28%) but higher than (0.92%) reported by (Emmanuel *et al.*, 2010). Higher moisture content of fruits has been established as a contributory factor to the microbial attack. Okudu *et al.* (2015) reported a moisture content of less than 10% (exactly 9.29%) which is comparable with the result of this study. Nwiisuator *et al*. (2012) observed that proximate contents were higher in the yellow arils compared with the seeds of *C. lepidota*, except for fats and carbohydrates. Both the fruits have very low amount of fat, 1.20% in *Cola lepidota* and 1.40% in *Cola pachycarpa* respectively. This type of food composition of low fat makes an ideal diet for overweight people.

A relatively appreciable level of proteins was found in both the species of fruits. The crude protein content of the seeds of *C. lepidota* and *C.pachycarpa* were observed to contain crude protein content of (25.62%) and (23.35%) respectively which are higher compared to the range (12.0% to 15.68%) reported by Emmanuel and Oludele (2010) for *C. citratus.* Thus, from the result, *C. lepidota* and *C.parchycarpa* could be a good source of protein. Protein is an essential component of diet which supplies adequate amount of amino acids.

Preliminary phytochemical analysis revealed the presence of phenols and also alkaloids, flavonoids, saponins, and tannins. These are important secondary metabolites since they play many biological roles (Trease and Evans, 2002). Alkaloid were reported in a similar investigation by Fabunmi & Arotupin (2015) to have pharmacological effects on humans and animals.

The study plants (*C.pachycarpa* and *C. lepidota*) were observed to have flavonoid content of 0.93mg/g) and 0.87mg/g respectively. These values are in agreement with the 0.49mg/g reported in a previous study by Iyawe and Azih, (2011). Flavonoid has antioxidant activity, and has also been demonstrated to be an effective anticancer promoters and cancer chemopreventive agents. Similarly, the tannin content of both species recorded 1.17mg/g and 1.08mg/g respectively for *C. parchycarpa* and *C. lepidota*, an observation that is slightly different for those reported by Ojo and Anibijuwon, (2010) for *C. citratus*. Tannins imparts astringent taste that affects palatability, reduce food intake and consequently growth. Tannins have been reported to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as proteolytic enzymes (Enwa *et al.*, 2014).

Okudu *et al.,* (2016) earlier reported flavonoids, saponins, phenols and tannins, which agrees with our report. However, they recorded and reported the highest quantity for alkaloids, which is in contrast to the report from this study.

Saponin content was observed in this study. This corroborates the studies of Ene-Obong *et al.*, (2016), who reported flavonoid and saponin as the most abundant phytochemicals in the fruit sample *C. parchycarpa* and *C. lepidota* recorded saponin content of 1.57mg/g and 1.49mg/g respectively. Saponins exhibit antioxidant and anti-inflammatory activity and are used in the management of hypercholesterolemia and hyperglycaemia (Oyinlade, 2014).

Again in this study, phenol was observed in both *C. parchycarpa* and *C. lepidota* which is in consonance with earlier report of Essien *et al.* (2015) where the highest phenol content was observed in the seeds (endocarp) of *C. lepidota*. 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 against heart diseases. Previous studies on this plant revealed presence of flavonoids, phenols and saponins (Ene-obong et al., 2016). These compounds have been reported to possess strong antioxidant capacity. The result show that the plant could be a

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

good source of antioxidant.

The existence of these phytochemicals in *C. lepidota* and *parchycapa* provides a basis for further development of this fruit into products to be used in disease therapy. However, *C. lepidota* and *parchycapa* do not have any antimicrobial activity on the clinical isolates of *Staphylococcus aureus* ATTC 6538, *Bacillus substilis* ATCC 6633 and *Escherichia coli* 0157:H7 **(**Abah *et al*., 2017) despite the presence of these phytochemicals which calls for further investigation. None of the three microbial species used in this investigation was susceptible to the two study plants at the concentrations tested. It could mean that the isolates were naturally resistant to the plants species or that the activities of the extract are plant parts dependent.

Both extracts of *C. lepidota* and *C.parchycarpa* in this study showed DPPH activity within the range (8.12 – 78.43 %) as reported earlier by Stojiljkovic *et al*. (2016). High scavenging activity of this extract can be explained on account of its high phenol and flavonoid content as has been described by Senguttavan *et al.* (2014). Thus, flavonoids have been proven to contribute to the DPPH scavenging activity of the extracts. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms. The EC50 values of scavenging DPPH radicals for the Cola *parchycarpa and Cola lepidota* were 11.8±0.08 and 12.8±0.08 mg /ml, respectively. This observation hence further adds to the existing data that these plant species have prominent antioxidant activity. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic (1.26mg/g in *C. lepidota* and 1.35.mg/g in C.*parchycarpa*) and flavonoid compounds (0.87mg/g in *C. lepidota* and 0.93.mg/g in C. *parchycarpa*). Our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity.

C. lepidota and C. *parchycarpa* fruits presented good antioxidant properties by the ABTS scavenging activity. The ABTS scavenging activity of *C. lepidota*was 82.2±0.58mmol TEAC/g) as against the 87.5±0.34mmol TEAC/g observed for *Cola pachycarpa*. The different plant species possessed strong ABTS scavenging activity an observation that is supported by earlier study by Sahreen *et al.* (2010).

Antioxidants are known to inhibit oxidation and oxidative stress, and remove potentially damaging oxidizing agents in a living organism, thus prevent diseases and serious ill-health in man and animals.

The antioxidant activity of phenols from these small wild fruits plays an important role in the protection of macromolecules from oxidative damage, thereby preventing many health problems including cancer, diabetes, cardiovascular diseases, and obesity (Paredes-Lopez *et al.*, 2010).

GC-MS analysis of the crude extract of *C. lepidota* seeds revealed that the main chemical molecules were Benzoquinone, 2,3-dimethyl and Squalene with retention time (RT) of 20.9 minutes respectively and 20.3 minutes respectively while the least was n-Hexadecanoic acid with retention time (RT) of 14.5 minutes. These compounds have been reported in earlier investigations including those of Olejnikova *et al.* (2009) and Jeon *et al.* (2005) to possess antioxidant, hypocholestrolemic and anti-inflammatory properties. Hexadecanoic acid, methyl ester and n-hexadecanoic acid have been reported to possess anti-inflammatory, antioxidant, hypocholesterolemic, 5-alpha reductase inhibitor, nematicide,pesticide and antiandrogenic (Praveen *et al*., 2010; Aleryani *et al*., 2005). 9, 12-Octadecanoic acid, methyl ester (linoleic acid methyl ester) also detected has been shown to possess remarkable anti-inflammatory, anti-histamine and anti-arthritics properties. It also possesses hepatoproctective and hypocholesterolemic properties (Henry *et al*., 2002).

In conclusion, this study was designed to analyze and compare the phytochemical constituent and antimicrobial properties of seeds of *Cola lepidota* and *Cola pachycarpa* to confirm their ethno-pharmaceutical claims. Therapeutically, the seeds are not found to inhibit against the selected clinical isolates which do not suggest good potential for use as antimicrobial and medicinal plant and as such not justified in their ethno-pharmaceutical uses in treatment of certain diseases. This aligns with work earlier conducted on the same plant fruit extract by Abah et al. (2017) where all the tested organisms were resistant to the extracts. Unlike the work conducted by Giwa et al. (2012) on *Cola miilenii* a similar plant seed and pulp extract which inhibited the isolates tested. Also the seeds of *C. lepidota* and *C. parachycha* contain a high amount of flavonoids and phenolic compounds, exhibit high antioxidant and free radical scavenging activities. The in vitro assays demonstrated that this plant extract is

a noteworthy resource of an innate antioxidant, which may help in preventing the development of diverse oxidative stresses. These results support the notion that a diet rich in herbs and plants can possibly reduce oxidation and act as a defence against associated disorders.

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