Investigate the Phytochemicals, Proximate Composition, Mineral Analysis, HPLC Components, and Antibacterial Activities of Crude Methanol Extract of Piper Guineense(Uziza) Seeds and Leaves

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ABSTRACT

The leaves and seeds of Piper guineense (Uziza) are widely used in homes to impact taste, aroma and flavor to food. In folk medicine, the Piper guineense seeds and leaves are used as Post- partum recovery after delivery. It is used in the enhancement of fertility in males and treatment of diabetes. Piper guineense is a wild shrub that has been adopted in homes and cultivated along the boundary fences. Therefore this study was aimed to determine the efficacy of crude methanol extract of Piper guineense seeds and leaves against pathogenic bacteria and fungi.The biotic components were estimated and its toxicity was determined using experimental albino rats. The seeds and leaves of Piper guineense was purchased from the open market and dried in the dark, grounded and stored in container till used. The ground powders were extracted using methanol via soxhlets apparatus. The extracts were used to estimate the phytochemical components, mineral and proximate properties. The bioactive compounds were determined using high performance liquid chromatography (HPLC) and Gas chromatograph-Gas spectrometry (GC- GM). The susceptibility patterns of the crude methanol extract were determined using agar diffusion technique for bacterial pathogens while macro-broth techniques were used to estimate the fungal pathogens. The toxic effect of crude methanol extract was investigated using 30-albino rats. The rats were group into five containing six (6) rats per group . Group A and B were administered with 500mg/ml body weight and 200mg/ml body weight of crude methanol seed extract while Group C and D were administered 1500mg/ml and 1000mg/ml per body weight of crude methanol extract of piper guineense leaves extract and group E were administered with normal saline and serve as net control. The experiment lasted for thirty (30) days. The haematological indices, Liver function test, Electrolytes and Urea were estimated while the visceral organs were harvested, fixed in 10 % formal saline and processed histologically using Haematoxylin & Eosin stain . The phytochemical components of Piper guineense seeds and leaves showed presence of soluble carbohydrates, alkaloids, tannins and saponins while proximate analysis indicated the high presence of carbohydrate and proteins in both seeds and leaves. Mineral composition includes sodium, calcium and phosphorus. Bioactive analysis of (uziza) leaves showed bioactive compounds; Resveratol, flavonones and malvidine while that of seeds included Ellagic acid, Resveratol and Quinine . The antibacterial activity of crude methanol extract of Piper guineense seeds and leaves both showed activity against Staphylocous aureus , proteus mirabilis and Escherichia coli while antifungal activity of these crude methanol extract inhibited the growth of Aspergillud flavus, Mucor fragilis and Penicillum notatum. The crude methanol fractions of the seeds were more potent in inhibiting both bacterial and fungal isolates. The crude methanol extracts had no effects on the hematological indices , liver function test and Aspartate Transaminase (AST) value in both seeds and leaves while the electrolyte ($Na+$, Ca^2+ and $Cl-$) showed increased values as compared to normal .Histological staining indicated that crude methanol extract of Piper guineense seeds affected the colon, Jejenum, liver and kidney with mild increase in inflammatory cells , liver necrosis while that of leaves, caused damage to colon , Jejunum inducing disruption and erosion of lining of crypts of liberkuhn. The findings in this study showed that crude methanol extract has good sensitivity pattern against bacterial and fungal pathogens. The extract, at higher dosages are toxic to the liver and colon, therefore its persistent use may lead to organ damage, hence the need to characterize the bioactive components and identify the toxic agents for elimination.

Keywords: phytochemicals, proximate, mineral analysis, guineense seed' crude methanol

INTRODUCTION

Medicinal plants plays important role in human health care system. These plants are natural and consist of roots, stem bark, leaves, flowers, seeds, fruits and those grouped as spices. Medicinal plants contain vital nutrients such as proteins, vitamins, minerals, carbohydrates, fibers and chemical components. Some of these plants are inform of spices and/or fruits, seeds and flowers that are useful to mankind. Piper guineense leaves and seeds are commonly used as spices due to its aroma and flavor impacts on foods. In folk medicine, the plant are used in treatment of diabetes, ulcer and enhances male fertility (Memudu, et al., 2015). Traditionally, both the leafy vegetables and seeds of Piper guineense are used as spices for preparing soup for post parturient women.(Udeh, et al., 1999). The plant is effectively used traditionally to terminate pregnancies under folk medicine practices (Iwu, 2014).

Piper guineense is a native of the tropics of western and central African regions and is common in southern Nigeria (Balofumi, et al., 2016). The plant belongs to Piperaceae family and of 20meters higher climbing vine with a peppery berry seeds. It is commonly known as West African black pepper. In Nigeria, Piper guineense has different local names like Uziza in Igbo, Iyere in Yoruba and Mosoro in Hausa.(Massawa, 2016; Mosaugo. et al., 2015: Uzodike and Onuoha 2015).

Microbial infectious agents are associated with antimicrobial usage for its cure. It is known that antibiotics and antifungal agents were developed to checkmate the spread of bacteria and fungi both in human population and her domesticated animals (WHO, 2014). The problems associated with the use of antibiotics and antifungal in treating microbial infections is high rate of resistance of these antimicrobial agents against the microorganisms. Multiple resistant organisms render therapy more precarious and costly and sometime unsuccessful. Individuals may be victim of multiple drug resistant infections because all available drugs have failed especially in developing world (Levy, 2002). This scenario presents a health challenge among the populace and thus the search for alternative antimicrobial agents especially from medicinal plants. Basically the wide folk medicinal use of leaves and seeds of Piper guineense within the populace is indication of its future application in the health system. Previously, it has been reported that the leaf methanol extract has antioxidant and protective effects on lead induced testicular damage in wistar rat (Nwosu, et al., 2022). It has also been shown that the leaves/seeds are used in the treatment of asthma, rheumatism and weight control (Jabeen, et al., 2010). Different authors has reported phytochemical, nutritional and antimicrobial properties of the plant based on different soil environments where the plants are collected (Chinwendu, et al., 2016; Uzokwe and Ezenwajugo 2023; Dingtsen, et al., 2020: Okoro, et al., 2020). Chinwendu, et al., reported the presence of alkaloids, tannins, saponins, flavonoids, hydrogen cyanides and phenols while Uzoekwe, et al., reported the phenols, tannins, Quinine and cardiac glycosides which are specific to the respective environment.

The seeds and leaves of Piper guineense have not been demonstrated to have toxic effect due to its use but rather more of its beneficial effects. However, according to a study by Madueke, et al., (2021), the results obtained suggest that Piper guineense seed may not be harmful at moderate dose; but high doses could be toxic in experimental animals.

Materials And Methods

Collection of Piper guineense leaves and seeds

Fresh leaves/Seeds of Piper guineense (African Black pepper) were bought at New Market Enugu, Enugu North Local Government area of Enugu State in June 2023. The leaves were carefully separated and sorted from their stalks. Both Piper guineense Leaves and Seeds were thoroughly washed with distilled water to remove dirt and microbial contaminant, sieved to removed excess water; Air-dried in the dark for three{3} weeks, grounded into fine powder using mill and stored in an air–tight plastic container for later use at room temperature.

Methanol Extraction

One hundred{100g} gram of the dried, grounded Piper guieneense seeds/leaves weighed out and wrapped in filter paper and then put in timber of soxhlet apparatus compartment, thereafter, the condenser/ heating mantel

was carefully and efficiently connected. The initial 500ml volume of the solvent {methanol} were added with the aid of the funnel by passing it through the timber containing the sample to the round bottom flask system of the soxhlet inlet and outlet of the condenser were connected to a host respectively for the recycling of cold water during extraction. Thereafter, the heat source was switched on about 5cm from the flask as the extraction process continued until the samples become lighter. The crude extract is then concentrated using water bath and the components characterized using standard methods {Okigbo, et al., (2010) and Nwankwo, et al., (2011).

Fractionation

The crude extract was adsorbed in a silica gel (70-230 mesh size] and removed fractionated using methanol fractionate (MF) which was used to increase the solvent polarities. The percentage yield was 16.75%. The crude extract was further fractionated using column chromatograph. Fifty (50) ml of the methanol extract was subjected to column chromatograph on silica gel (100-200 mesh merck) parked and eluted, therefore pure methanol crude extract was obtained.

Collection of Microbial Isolates:

Bacteria Isolates from different clinical samples were used for the susceptibility test. The bacteria included Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Klebsiella oxytoca, Streptococus pyogenes, Pseudomonas aeroginosa, Proteus mirabilis.

Agar well diffusion technique was used for susceptibility pattern of the extracts. Briefly, the crude methanol extracts of the seeds and the leaves were separately diluted in 10% concentration using Dimethyl sulphuroxide (DMSO). The extract was further diluted to 100µg/ml which is the working concentration. The crude methanol extract 100ug/ml was serially diluted from 100 µg`/ml to 0.195ng/ml in test tubes using DMSO. The diluent was used because the extract could not dissolve in sterile water. The selected bacterial Isolates were prepared by inoculating the organisms into peptone water, incubated at 37ºC for between one hour in case of fast growing bacteria (Escherichia coli, Klebsiella pneumonie, Proteus mirabilis) to three (3) hours in case of slowing bacteria; Staphylococcus aereus and Streptococcus pyogenes.

METHODOLOGY

Reagents include urea acid reagent (Reagent No 68) and Diacetylmonoxime, 4g/l (reagent No 21) which can be stored at room temperature. Equal volumes of urea acid regent and diacetyl monoxime reagent were mixed. Allowed 4mls of colour reagent for each tube. Speamen ÷ 20µl (0.02ml) or rabbit serum.

Tubes were rebelled accordingly.

B – Reagent Blank

S – Standard, 10mmol/L

L control Serum

Other tubes l to say 30 according to number of samples 4ml of freshly made urea colour (highly corrosive) were pipetted into each tube. Each tube was Gödel 20ml (0.02ml) distilled water for B, 20µl standard (10mmol/L); 20ul control serum to C and 20µL of rats serum to other tubes.

The contents of each tube were mixed well and incubated at 100ºC for 15 minutes on a heat –block set at 100ºC each tube was stoppered using loose flitting cap. The tubes were removed and cooled the contents in a container of coloured water for about 5miuntes without allowing water to enter the tubes nor allowed exposure to light for I hour he absorbance's of the solutions in a colorimeter were read using a green filter 520nm (eg 11 ford No 604) (or a spectrophotometer set at wavelength530nm. The instrument was zeroed with a blank solution (Tube B). The concentration of urea in the control and rat's samples can be calculation. By reading the values from the calibration graph providing the reading of the 10mmol/L stand and agree with the calibration or using the formular

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$$\text{Urea mmol/L} = \frac{AT}{AS} \times 10$$

Where AT =Aabsorbance of test(s) or control

AT = Absorbance of 10 mmol/L standard

Estimation of Serum Bilirubin

The measurement of serum or plasma is usually performed to investigate the causes of liver disease and jundice.

Principle of Jendrassik and Grof bilurubin method

Sulphanilic acid is diazotized by the nitrous acid produced from the reaction between sodium nitrite and hydrochloric acid.

Bilirubin reacts with the diazotized sulphanilic acid (diazo reagent) to form azobilirubin caffeine is an accelerator and gives a rapid and complete conversion to azobirubin. The pink acid azobilrubin is converted to blue azobilirubin by an alkaline tartrate reagent and the absorbance of the blue –green solution is read in calorimeter using an orange filter 590nm (11 ford) No 607 or in a spectrophotometer at wave length 600nm. Conjugated (direct) bilirubin. This is measured in the absence of the caffeine – benzoate catalyst and at an acid pH. Urea these conditions, only the conjugated bilirubin will react the reaction is terminated by ascorbic acid and alkaline tartrate is added.

Materials

Sulfanilic acid 5g/dl (Repent No 65), Sodium nitrite 5g/L (Regent No6), Caffenine –Sodium benzoate (Regent No 16) and Alkaline tartrate reagent (Regents).

All the reagents except the sodium nitrite reagent are stable at room temperature (20-29ºC) for about 6 months. The sodium nitrite reagent must be stored at 2-8ºC. It is stable for at least 1 month when kept tightly stopper.

Diazo Reagent: Mix 20.0ml sulphanilic acid reagent with 0.5ml sodium nitrite reagent.

Diazo reagent is stable when kept tightly stoppered at 2-8ºC.

Method

Tubes were labeled according to sample numbers as follows

S- Standard, SB

SB – standard Blank

C – Control Serum

CB – Control Blank

1,2 – Rats tests

1b2b 5- rat's blank

1ml of caffeine benzoate regent was pipette into each tube and to each tube as follows

C.SB – O. 1 ml standard serum (170-340 µmol/L)

C,CB. 0.1ml control serum

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XII Issue X October 2025

1;1B, 22blank -0. 1ml rat serum

The contents were mixed well 0.5m of diazo reagent was added was to tubes, 5,C,1,2 5, added well, added 0.5ml of sulpharlic acid reagent to tubes 5B, CB, 1B, 2B-5B and mixed were. The mixtures were left at room temperature at 25ºC for 5 minutes: 1ml of alkaline tartrate reagent was added to each tube and mixed well (which cleans any turbidity). The absorbance's of the solutions (blank first) in the colorimeter using orange files, 590nm eg 11ford No 607 or in a spectrophotometers set at wavelength 600nm, was read while the colorimeter was zeroed with distilled water .Calculate the concentration of the total bilurubin in the control and rats samples.

RESULTS

Piper guineense

The seeds and leaves of Piper guineense were extracted using methanol and tested for anti-bacterial and antifungal properties. The bacterial and fungal isolates were obtained from clinical samples at Medical Microbiology, University of Nigeria Teaching Hospital Ituku- Ozalla, Enugu. These included Staphlococcu aureus, Proteus mirabilis, Klebsiella pneumonia, Klebsiella oxyitica, Pseudomonas aeroginosa, Streptococcus pyogenes, Escherichia coli, Mucor fragilis,Aspergillus niger, Aspergilus flavus, Aspergillus fumigatus, Penicillum notatum,Trichophyton soudense and Candida albican (Table 1).

Anti-bacterial activity of crude methanol extract of Piper guineense (seeds)

The efficacy of the crude methanol extract of the seed was more against Staphyloloccus aureus, Klebsiella pneumonia and Klebsiella oxytica at a concentration of 3.125 mg/ml with zone of inhibition of 10mm in diameter. Also the extract was effective against Pseudomonas aeuroginosa, Streptococcus Pyogenes and Escherichia coli at concentration of 6.25mg/ml. The least organism that crude methanol extract was effective was Proteus mirabilis which was sensitive to the extract at 25mg/ml with a zone of inhibition of 8mm in diameter.(Table 4.2)

Antibacterial activity of crude methanol extract of Piper guineense (leaves).

The crude methanol extract of the leaves extract was effective against Staphylococcus aureus and Klebsiella oxytica at a concentration of 3.125mg/ml with zone of inhibition of 10mm in diameter. The methanol extract of Piper guineensewas effective against Klebsiella Pneumonia,, Pseudomonas aeroginosa, Staptococcus pyogenes and Escherichia coli at a concentrator of 6.2 5m/ml with different zones of inhibition with Klebsiella Pneumonia given higher zone of inhibition of 15mm in diameter against 10mm of others(Table 4 3)

TABLE 1.O: Sources of microbial isolates for Piper guineense activity.

Bacterial/Fungal Isolate	**Sources**
Staphylococcus aureus	Wound swab
Proteus mirabilis	Urine
Klebsiella pneumonia	Sputum
Klebsiella oxytica	Urine
Pseudomonas aeuroginosa	Wound swab
Steptococus pyogenes	Ear swab
Escherichia coli	Urine
Mucor fragilis	Hand (ulna)
Aspergillus niger	Stomach
Aspergillus flavus	Hair (head)

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XII Issue X October 2025

Aspergillus fumigatus	Sputum
Penicillium notatum	Hand
Trichophyton souduense	Foot nail Scrapping
Candida albicans	High vaginal swap

Table 1.1: Antibacterial activity of crude Methanol extract of Piper guineense (seeds).

Bacteria isolates	100(mg/ml)	50	25	12.5	6.25	3.125	1.5625	0.781	0.391	0.1953
Staphylococcus aureus	20mm	15mm	15mm	15mm	10mm	10mm	-	-	-	-
Proleus mirabilis	12 mm	20mm	8mm	-	-	-	-	-	-	-
Klebsiella pneumonia	20 mm	20	18	18	10	10	-	-	-	-
Klebsiella oxytica	20	20mm	20mm	18mm	15mm	10mm	-	-	-	-
Pseudomonas aeroginosa	25mm	25mm	20mm	15mm	10mm	-	-	-	-	-
Steptococous pyogenes	20mm	18mm	16mm	15mm	10mm	-	-	-	-	-
Escherichia coli	20mm	20mm	18mm	15mm	10mm	-	-	-	-	-

Table 1.2: Antibacterial activity of Piper guineense crude Methanol extract of leaves

Bacteria isolates	100mg/ml	50	25	12.5	6.25	3.125	1.5625	0.781	0.391	0.1953
Staphylococcus aureus	20mm	20mm	15mm	15mm	10mm	10mm	-	-	-	-
Proteus mirabilis	13 mm	12mm	10mm	-	-	-	-	-	-	-
Klebsiella pneumonia	20 mm	20mm	20mm	18mm	15mm	-	-	-	-	-
Klebsiella oxytica	28	25mm	25mm	22mm	20mm	10mm	-	-	-	-
Pseudomonas aeroginosa	22mm	20mm	15mm	10mm	10mm	-	-	-	-	-
Steptococous pyogenes	20mm	15mm	15mm	10mm	10mm	-	-	-	-	-
Escherichia coli	25mm	22mm	20mm	18mm	10mm	-	-	-	-	-
No zone of inhibition -										

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XII Issue X October 2025

Antifungal activity of crude methanol extract of Piper guineense (seeds)

The crude methanol seed extract inhibited the growth of Mucor fragilis, Aspergillus niger, Aspegillus fumigatus, at a concentration of 12.5mg/ml after 21 days of incubation. The Candida albicans was inhibited at the concentration of 6.25mg/ml while Trichophyton soudense was inhibited at 12.5mg/ml.(Table1.3)

The crude methanol extract of Piper guineense (leaves)

The leaves crude methanol extract of Piper guineense had inhibitory activity against Aspergillus niger, Mucor fragilis , Candida albicans at a concentration of 3.12mg/ml while showing inhibition effect at higher concentration against Penicillium notatum, Trichophyton soudanense at a concentration of 12.50 mg/ml while it has effect on Penicillium notatum at concentration of 25mg/ml after 21 days of incubation. At the same of 25mg/ml, The Piper guineense leaves extract inhibited Aspergillus fumigatus at a concentration of 6.25mg/ml.(Table 1.4)

Activity of Piper guineense methanol fractions

Activity of Piper guineense methanol fractions (seeds)

The methanol fractions of the Piper guineense seeds was effective against Staphylococcus aureus, Klebsiella oxytica, Pseudomonas aeuroginosa at a concentration of 3.125mg/ml with zone of inhibition of 10mm respectively Proteus mirabilis, Klebsiella pneumonia, Streptococcus pyogenes and Escherichia coli were controlled by the fraction at a concentration 6.25mg/ml..(Table 1.5)

Table 1.3: Anti-Fungal activity of crude seed methanol extract of Piper guineense.

Fungal isolates	100mg/ml	50	25	12.5	6.25	3.125	1.5625	0.781	0.391	
Mucor fragilis	-	-	-	-	+	+	+	+	+	+
Aspergillus niger	-	-	-	+	+	+	+	+	+	+
Aspergillus flavus	-	-	-	-	+	+	+	+	+	+
Candida albicans	-	-	-	-	-	-	+	+	+	+
Aspergillus fumigatus	-	-	-	-	+	+	+	+	+	+
Penicillium notatum	-	-	+	+	+	+	+	+	+	+
Trichophyton Soudanense	-	-	-	+	+	+	-	+	+	+
No Growth after day 21days - Growth after day 21days +										

Table 1.4: Anti-fungal activity of crude methanol extract of Piper Guineense Leaves

Fungal isolates	100mg/ml	50	25	12.5	6.25	3.125	1.5625	0.781	0.39
Aspergillus niger	-	-	-	-	-	+	+	+	+
Aspergillus flavus	-	-	-	-	-	-	+	+	+

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XII Issue X October 2025

Mucor fragilis	-	-	-	-	-	+	+	+	+
Candida albicans	-	-	-	-	-	+	+	+	+
Aspergillu sfumigatus	-	-	-	-	+	+	+	+	+
Trichophyton soudanense	-	-	-	-	+	+	+	+	+
Penicillium notatum	-	-	-	+	+	+	+	+	+

Table 1.4: Anti-bacterial activity of fractionation extract of Piper guineense (Uziza)seeds

Bacteria isolates	100	50	25	12.5	6.25	3.125(mg/ml)	1.5625	0.781	0.391	0.1953
Staphylococcus aureus	32mm	30mm	30mm	28mm	20mm	10mm	-	-	-	-
Proleus mirabili	20 mm	18mm	15mm	15mm	10mm	-	-	-	-	-
Klebsiella pneumonia	25 mm	22	20mm	18mm	10mm	-	-	-	-	-
Klebsiella oxytica	30	25mm	20mm	15mm	10mm	10mm	-	-	-	-
Pseudomonas aeruginosa	32mm	30mm	28mm	25mm	20mm	10mm	-	-	-	-
Steptococous pyogenes	25mm	22mm	20mm	18mm	10mm	-	-	-	-	-
Escherichia coli	25mm	22mm	20mm	15mm	8mm	-	-	-	-	-

Activity of Piper guineense methanol fraction (leaves) .on bacterial isolates.

The antimicrobial activity of the Piper guineense leaves of crude methanol fraction inhibited the growh of Staphylococcus aureus and Klebsiella pneumonia; Klebsiella oxytiaa and Eschicheria coli at a concentration of 3.125mg/ml with at 10mm and 15mm in diameter respectively. (Table 1.5)

Activity of Piper guineense seeds and leaves fractions on fungal isolates.

Both the Piper guineense leaves and seeds fractions inhibited the growth of Mucor fragilis, Aspergillus niger, Aspergillus flavus, candida albicans, Aspergillus fumigatus and Penicillum notatum..(Table 1.8)

Table 1.6: Anti-bacterial activity of fractionation extract of Piper guineense (uziza) Leaves

Bacteria isolates	100	50	25	12.5	6.25	3.125	1.5625	0.781	0.391	0.1953
Staphylococcus aureus	30mm	28mm	25mm	20mm	15mm	10mm	-	-	-	-
Proleus mirabilis	30mm	25mm	20mm	18mm	15mm +		-	-	-	-
Klebsiella pneumonia	25mm	22mm	20mm	20mm	15mm	10mm	-	-	-	-

Klebsiella oxytica	33mm	30mm	28mm	25mm	22mm	15mm	-	-	-	-
Pseudomonas aeuroginosa	28mm	25mm	25mm	22mm	15mm	-	-	-	-	-
Steptococous pyogenes	25mm	20mm	20mm	15mm		-	-	-	-	-
Escherichia coli	32mm	30mm	28mm	25mm	20mm	15mm	-	-	-	-

Table 1.7: Anti-fungal activity of fraction extract of Peper guineense(Uziza) Seeds and leaves

Fungal isolates	**Leaves**	**Seeds**
Mucor fragilis	Inhibition	Inhibition
Aspergillus niger	Inhibition	Inhibition
Aspergillus flavus	Inhibition	Inhibition
Candida albicans	Inhibition	Inhibition
Aspergillus fumigatus	Inhibition	Inhibition
Penicillium notatum	Inhibition	Inhibition

Table 1.8: Mean Weights of rats before-and-after exposure to Piper guineense crude methanol extract

Group	**Before (M+SD)g**	**After (m±SD)g**	**T**	**P-value**	**Remarks**
A	95.23±12.37	124.73±10.32	-4.924	0.004*	Significant
B	99.23±9.31	107.82±48.17	-0.441	0.678	Not Significant
C	162.12±17,49	158.48±21.08	0.318	0.768	Not Significant
D	105.06±5.32	142.68±12.13	-5.610	0.005*	Significant
E	95.52±19.03	123.45±16.21	-7.692	0.001*	Significant

Effects of Crude Methanol Extract (seeds) on hematological indices.

The damaging effect of crude methanol extract of Piper guineense indicated that packed cell volume (PCV), hemoglobin (Hb) and Red blood cells (RBC) were not affected by the seeds and leaves of methanol extract though there were minor increases of the PCV and Hemoglobin compared with the control.

The methanol leaves extract administered at 500mg/kg weight indicated that PG was 53± 2.86% (percent) when compared with the control which 47.8±16% in the same way the leaves administered at 1500 mg/kg increased hemoglobin content to 194±14.07gldl when compared to the control which is 158.5±3.2gldl. the total white blood cell (TWBC)were affected by the crude methanol leave extract when compared with that of the seeds.

The TWBC was lowered in leaves in the experimental rats.1500mg/ml and 100mg/ml with $5.92\pm2.99x10^5$ and $5.81\pm0.99x10^5$ which was statistical significant when compared with the seed that received 500mg/ml. It was observed that seeds receives 200mg/ml of the extract has a mass reduction of TWBC 3.69±0.92. All the TWBC were statistically significant when compared with the control. The lymphocytes showed that marked reduction in Group A and Group B that were administered with seeds while compared with the methanol leaves extract:

The seeds and the leaves extracts administered showed platelet levels when compared with the control (E).(Table4. 10)

The phytochemical content of methanol extract of Piper guinense

The phytochemical properties of the Piper guineense of seeds and leaves were alkaloids, flavonoids, terpernoids, total phenol, soluble carbohydrate, tanins and Saponins Cardiac glycosides were absent from both. The concentration of these phytochemical components varied greatly between the leaves and seeds for example the soluble carbohydrates with 18.365±0.233mgl 100g was detected in the seeds were as in leaves, it was 11.285±0.063.

The presence of alkaloid and flavonoids and terpenoids were estimated at 8.28±0.10, 5.and 11.49±0.22mg/100g in the seeds while the concentration in leaves were 4.81±0.18, 7.03±5.55±0.01 respectively. Total phenol, tannins and Saponins were higher value in the leaves than the seeds.(Table 4.13)

Table 1.9: Estimation of electrolytes and urea levels in the experimental rats.

GROUPS	SERUM SODIUM (MMOL/L)	SERUM POTASSIUM (MMOL/L)	SERUM CHLORIDE (MMOL/L)	SERUM CARBONATE (MMOL/L)	SERUM UREA (MMOL/L)
Group A	13.9±0.0	5.4±1.31	103.55±3.67	22.54±6.22	8.75±1.12
Group B	116.49±3.40	5.5±1.20	100.33±6.51	19.81±1.54	8.01±3.33
Group C	127.2±15.1	6.1±1.17	103.87±5.11	21.59±10.83	9.15±2.86
Group D	139.50±14.24	5.3±1.9	113.57±9.50	22.25±6.63	10.69±1.21
Group E	116.3±14.14	5.7±1.05	95.7±7.56	16.73±0.43	7.00±0.3

Table 2.0: Phytochemical properties of Piper guineense (Uziza) seeds and leaves

Components	Seed (mg/100g)	Leaves (mg/100g)
Alkaloids	8.28±0.098	4.805±0.176
Flavonoids	5.795±0.049	7.025±0.120
Terpenoids	11.485±0.219	5.55±0.014
Total phenolic	7.74±0.028	12.75±0.014
Soluble Carbohydrate	18.365±0.233	11.285±0.063
Tannins	1.89±0.042	5.03±0.208
Saponins	2.21±0.141	11.72±0.226
Cardiac glycoside	-	-

The Proximate content of methanol extract of Piper guineense seeds and leaves

The proximate analysis that indicated high concentration of carbohydrate in both the seeds and the leaves constituting 62.18±0.44% and 53.61±0.69% respectively. The fat content was higher in the seeds than the leaves 2.01±071% in the seeds and leaves respectively. In other proximate constituents moisture, fibre and proteins were more in eaves than the seeds (Table 4.14)

The mineral content of methanol extract 0f Piper guineense seeds and leaves.

The mineral components of Piper guineense of seeds and leaves. Showed that potassium, 92.81±0.52, Sodium,

51.54±0.39.Phosphorus, 17.65±0.56; mg/100g were higher in the seeds than the leaves 85.74±0.15mg/00g;47.76±1.29mg/100g, 15,15±1.29mg/100g while calcium; magnessuim and iron were higher values in the leaves than the seeds. The concentration of calcium was 250.98±8.97 mg/100g while in the seeds, it was 172.65±5.49mg/100g. The content of magnesium was 64.82±0.00 mg/100g as in the seeds as against 149.78±0.05 in the Leaves.(Table4.15)

Table 2.1: Proximate analysis of Piper guineense(Uziza) seeds and leaves

Components (%)	**Seeds**	**Leaves**
Moisture (%)	7.505±017	6.195±0.044
Fat (%)	5.93±0.14	2.01±0.070
Ash (%)	7.04±0.03	7.04±0.075
Fibre (%)	6.3±0.11	17.125±0.33
Protein (%)	11.05±0.28	13.7±0.365
Carbohydrate (%)	62.18±0.44	53.61±0.691

Table 2.2: Mineral components of Piper guineense seeds and leaves

Types of mineral (mg/100g)	**Seeds**	**Leaves**
Sodium	51.54±0.39	47.76±1.29
Potassium	02.81±0.52	85.74±0.15
Magnesium	64.82±0.00	149.78±0.05
Iron	22.64±0.04	31.26±0.05
Calcium	172.65±5.49	250.99±8.97
Phosphorus	17.65±0.56	15.15±1.29

Chemical Composition of the Piper guineense seeds and leaves.

The Gas chromatograph-Mas spectrometry (GC-MS) was performed to elucidate the chemical compounds of Piper guineense seeds and leaves. The leaves had more of Dedeconic acid which were detected at different level of the Retention time such as 15.018 and 16.539, 16.736 respectively. Other chemical components include 9-hexadecanoic acid and trans-3 ethoxy-6methyl/ Oleic acid was also obtained at different retention time. Also present was ethanolic and alpha-d-glucose..

The chemical composition of the leaves also showed high presence of dedocanoic acid which were obtained at different retention time ranging from 7.36 to 17.78/ seconds. Other chemicals present were methoxyacetic acid which appeared in three (3) different retention time Benzamide were also obtained at two (2) different retention time (RT) while Hexadecanoic acid, Diethylene glycol momododecyl esther and Urucic acid were present.(Table2.3)

Table 2.3: Chemical components of crude methanol Piper guineense seeds and leaves (Gc-ms)

		SEEDS		LEAVES	
S/N	COMPOUNDS	RT	AREA	RT	AREA
1	2- Thiazoline	6.567	0.01		
2	Alpha-phellandiene	7.102	0.02		
3	Benzeanamine	7.524	0.02		

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XII Issue X October 2025

4	Oleic acid	7.665	0.01		
5	Trans-3 Ethoxy b-methyl-b-nitostyrene	7.863	0.01		
6	Oleic acid	8.510	0.01	10.849	0.06
7	9 Hexadenoic acid	8.623	0.00		
8	1- Docosene octadesone	9.778	0.02		
9	1 Docosene	9.891	0.00		
10	E Beta – famesene	11.778	0.22		
11	1- Hexacosene	13.694	37.54		
12	Hexadeconoic acid	14.285	3.23	12.849	1.93
13	Alpha –d glucose	14.539	4.18		
14	Dodeconoic acid	14.652	3.70	7.355	0.00
15	Dodeconoic acid	14.849	2.24	8.539	0.03
16	Dodeconoic acid	15.018	3.55	13.666	8.60
17	Dodeconoic acid	15.412	7.57	13.975	6.97
18	Dodeconoic acid	15.919	5.01	14.285	8.64
19	Ethanol 2 – (octadelyl-oxy)	16.539	10.36		
20	Octadecane	16.736	19.12		
21	Alpha d- glucose	17.638	2.37		
22	Alpha d- glucose	18.201	0.20		
23	Dodeconoic acid	18.426	0.64	14.652	8.63
24	1 – propene			7.665	0.00
25	9 – Tricosene			9.412	0.01
26	Cyclohexasiloxane			10.229	0.02
27	Benzamide			11.553	0.13
28	Erucic acid			11.806	0.08
29	Methoxyacetic acid			15.074	9.40
30	Hexadecane			15.187	2.95
31	Methoxyacetic acid			15.299	4.86
32	Diethylene glycol monododecyl ether			15.525	12.12
33	Methoxyacetic acid			15.750	9.20
34	Methoxyacetic acid			15.947	8.21
35	Dodeconoic acid			16.229	7.09
36	4 – Dibenofura namine			13.666	8.60
37	Dodeconoic acid			16.708	2.92
38	Dodeconoic acid			17.018	1.41
39	Dodeconoic acid			17.102	0.67

40	Dodeconoic acid			17.299	1.22
41	Dodeconoic acid			17.384	2.35
42	Dodeconoic acid			17.778	0.59
43	Benzamide			18.511	2.24

Table2.4: Bioactive components of crude methanol extract of Piper guineense seeds and leaves (HPLC)

		CONCENTRATION	
S/N	BIOACTIVE COMPOUND	SEEDS (ug/ml)	LEAVES (ug/ml)
1	Resveratol	10.9048	39.2350
2	Proanthocyanidirus		85.48115
3	Flavonones		20.2307
4	Delphionidin		42.8065
5	Pyranphanthocyanin		6.8082
6	Alglycone		25.0240
7	Anthocyanidimes		74.6359
8	Malvidine		209143
9	Epiheridrine	12.0701	
10	Ribalinidine	12.6048	
11	Ellagic Acid	63.4744	
12	Spartein	13.3032	
13	Naringin	36.9169	
14	Lunamarin	11.4862	
15	Qunine	60.0592	
16	Kaemferol	4.2534	
17	Naringnin	40.5806	
18	Rutin	12.0353	
19	Quinine	44.0298	

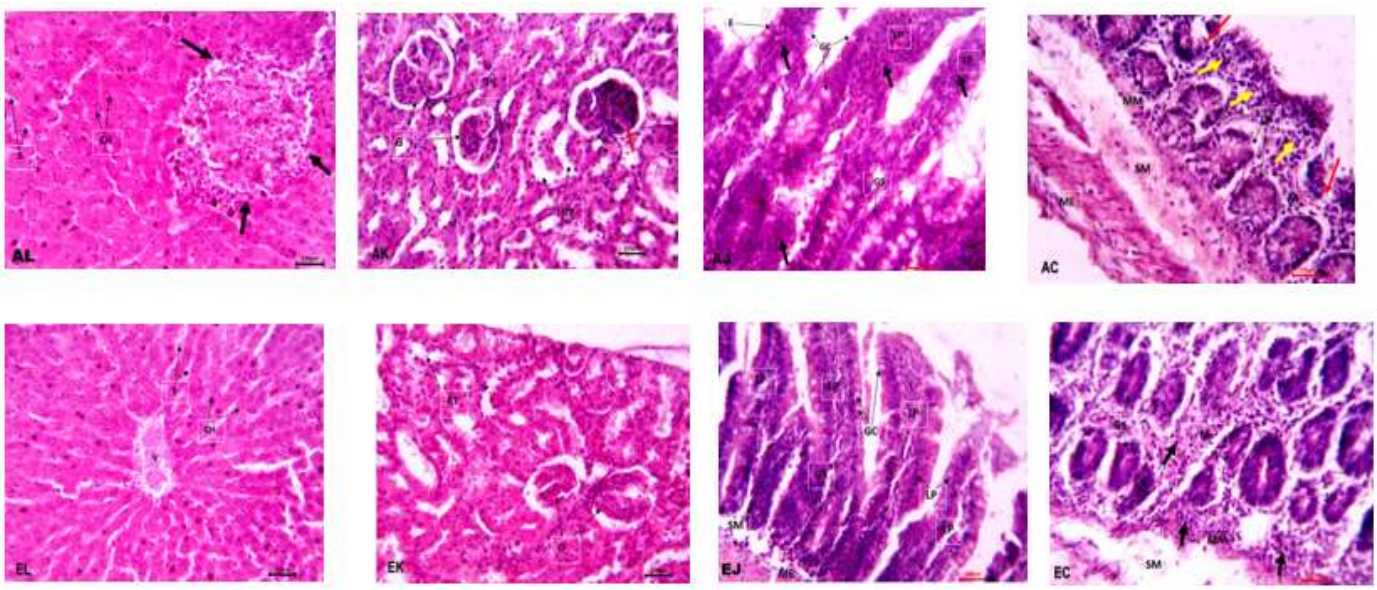
Photomicrographs Hematoxylin and Eosin (H&E stain) illustrating tissue sections from Group A rats (AL, AK, AJ, AC) showing varying degrees of histological alterations compared to their normal controls (EL, EK, EJ, EC). In Group A, the liver section exhibited focal necrosis with mild inflammatory infiltration; the kidney section showed features suggestive of glomerulosclerosis; the jejunum revealed moderate inflammatory cell infiltration and increased goblet cell numbers; and the colon showed slight epithelial disruption and moderate lamina propria inflammation. In contrast, the control group demonstrated normal tissue architecture across all examined organs.(Fig 1.0)

Photomicrographs (H&E stain) demonstrating tissue sections from Group B rats (BL, BK, BJ, BC) exhibiting varying degrees of histopathological changes compared to the Normal Control (EL, EK, EJ, EC). In Group B, the liver section showed piecemeal necrosis with moderate inflammatory infiltration; the kidney exhibited marked tubular atrophy, interstitial fibrosis, and mild interstitial inflammation; the jejunum revealed relatively preserved architecture with mild lamina propria inflammatory infiltration; and the colon displayed nearly normal crypts but with increased mucosal cellularity, edematous lamina propria, and thickened muscularis mucosa. In contrast, the control group maintained normal histological architecture across all tissues.(Fig1.1)

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XII Issue X October 2025

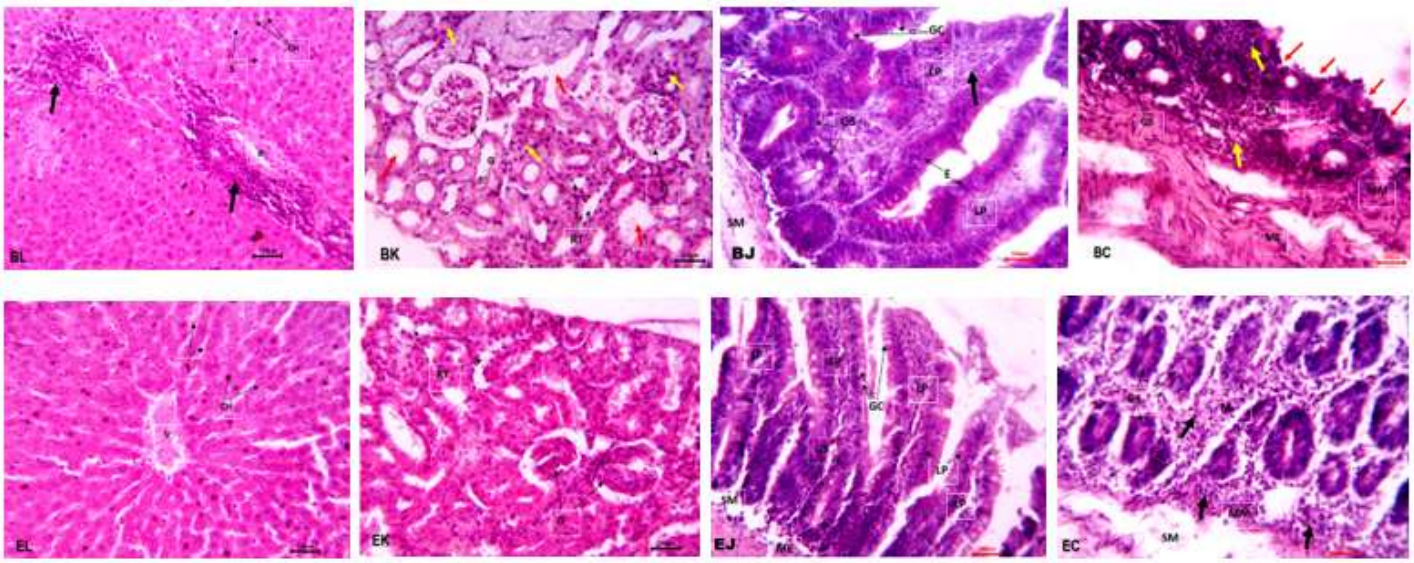
Group A

Figure: 1.0… Photomicrographs Comparing Histological Changes in Liver, Kidney, Jejunum, and Colon Tissues of Albino Rats in Experimental Group A and Normal Control



Group B

Figure 1.1….: Photomicrographs Comparing Histological Changes in Liver, Kidney, Jejunum, and Colon Tissues of Albino Rats in Experimental Group B and Normal Control



Photomicrographs (H&E stain) illustrating tissue sections from Group C rats (CL, CK, CJ, CC) showing histopathological alterations compared to the Normal Control group (EL, EK, EJ, EC). In Group C, the liver section displayed multiple areas of necrosis with inflammatory cell infiltration; the kidney revealed mild tubular dilation, proteinaceous material accumulation, edematous interstitium, and inflammatory infiltration within Bowman's space and interstitium; the jejunum showed villous atrophy and moderate inflammatory infiltration within the lamina propria; and the colon demonstrated relatively preserved architecture with mild inflammatory infiltration in the lamina propria. In contrast, the Normal Control group maintained normal histological features across all examined tissues.(Fig 1.2)

;Sections from experimental rats (DL–DC) showing hepatic necrosis, renal tubular alterations, jejunal inflammatory infiltration, and mild colonic inflammation. Normal controls (EL–EC) display preserved tissue architecture. (H&E stain, magnifications as indicated).

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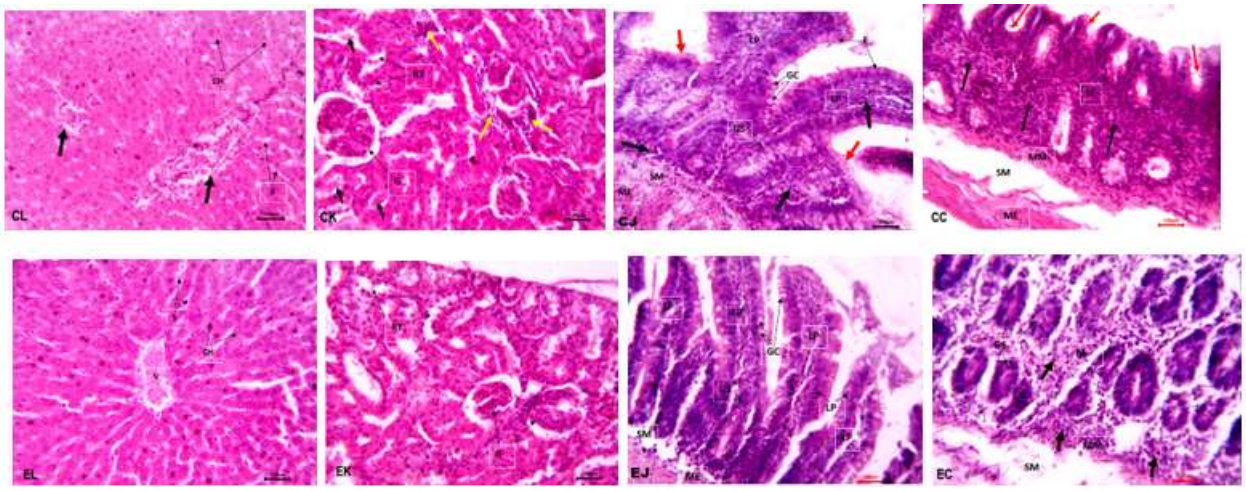
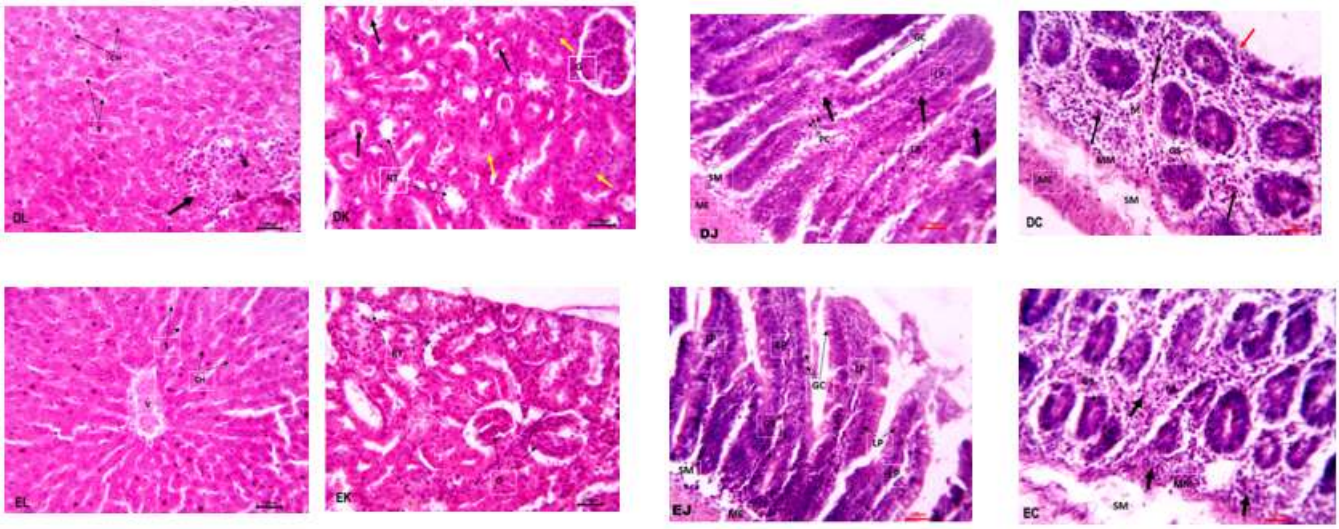
Group C

Figure 1.2…: Photomicrographs Comparing Histological Changes in Liver, Kidney, Jejunum, and Colon Tissues of Albino Rats in Experimental Group C and Normal Contro



Group D

Figure 1.3…. : Photomicrographs of the liver, kidney, jejunum, and colon of albino rats showing histological alterations in the experimental group compared to the normal control group



DISCUSSION

The spice, Piper guineense is a well adored plant in South East Nigeria due to its flavor and adding taste to food. In some localities, then Piper guineense seeds and leaves are used for treatment of malaria, respiratory infections and aphrodisc (Ekanem,and Obiekezie 2000; Morrissey, et al.,1999). The leaves in particular are used as preparation for postpartum woman to encourage uterine involution (Udoh, 1999). It has antifertility effects (Ekanem and Obiekezie, 2000) as well as anticonvulsant effect (Abila, et al., 1993). Therefore, the seeds and leaves of Piper guineense may have both beneficial effect and adverse effect on humans. Thus, this study aims to determine the activity of crude methanol extract of Piper guneense seeds and leaves on bacterial and fungal agents. Secondly, the biochemical components and its toxicity on experimental albino rats were studied. The crude methanol extract of Piper guineense seeds had varying effects on pathogenic bacterial isolates. The zone of inhibition exhibited ranges from 8mm in diameter to 20mm in diameter among all the bacteria at a concentration of 3.1 mg/ml to 100mg/ml. This was in agreement with the report of Udoh, Akpan and Ufaruma (1996);Udoudoakpan and Effion (2024) that ethanol and aqueous extract of the seeds of Piper guineense had a wide range of killing effect of bacterial isolated from bread..Ogumefua, et al.,(2017) reported that aqueous extract of Piper guineense seeds, Ethanol extract of seeds, and n-hexane of the seeds extract of Piper guineense had inhibitory effect on bacterial at the range of 3-29mm, 4-22mm and 7- 14mm in diameter. This indicated

that Piper guineense seeds can be used in the control of common pathogens. The methanol of the leaves showed a good killing effect against pathogenic bacteria that was tested. The zone of inhibition decreased as the concentration decreases and at a concentration of 100mg. the highest zone of inhibition was observed to be 20mm in diameter while the least at the concentration of 3.125mg/ml at 10mm in diameter for Staphylococcus aureus. Thus the zones of inhibition of the crude methanol extract of Piper guineense ranges from 28mm to 10mm (Klebsiella oxytica) in diameter while the least efficacy was observed in Proteus mirabilis with a range of 13mm-10mm in diameter at a concentration of 25ml/ml which agreed with Mgbeahuruike, et al., (2018), Who showed Proteus mirabilis zone of inhibition at 16mm in diameter but disagreed with Okeke, et al., [2001] who reported that Piper guineense seeds was effective to Proteus vulgaris but not to Proteus mirabilis.

The crude methanol extract of Piper guineense seeds and leaves had effects on common bacterial isolates which can be explored for treatment of human illness. The mechanism of this killing effects were based on disrupting their cell membrane and other vital processes for instance Tannis have been found to form irreversible complexes with proline rich protein (Shamada 2006) resulting in inhibition of cell protein synthesis. Parekh and Chanda, [2007] reported that taninis are known to react with protein to provide typical tanning effect. .Piperine (alkaloids) had shown to have properties of antibacterial activity Heinrich et al.,2021).

The crude methanol seeds extract showed inhibitory effects against all fungi at a concentration of 100mg/ml. Mucor fragils, Aspergillus flavus, Candida albicans and Aspergillus fumigatus had the highest inhibitory effect at 25mg/ml while the least inhibitory was Penicillum notatum at concentration of 50mg/ml.

The crude methanol extract of Piper guineense leaves had similar effect when compared to the seeds as in Aspergillus niger, Aspergillus flavus, Mucor fragilis and Candida albicas were inhibited at concentration of 6.25mg/ml while Penicillum notatum was least at concentration of 50mg/ml. The overall effect of crude methanol extract of Piper guineense seeds and leaves seems to be minimal on fungal agent. It may be suggested that the leaves and seeds of Piper guineense may be used in combination with other medicinal plant to achieve maximum effect. The crude methanol extract fractions of the seeds showed killing effects against bacterial pathogens at concentration of 100mg/ml. Staphylococcus aureus was inhibited at a range 32mm-100mm in diameter while the least inhibited was Proteus mirabilis. Escherichia coli (E coli) growth was inhibited at a range of 25mm-8mm in diameter Pseudomonas aeroginosa was inhibited at 32mm. Pseudomonas aeroginosa, Klebsiella oxytica, Klebsiella pneumonia 30mm-10mm and I0mm-1omm respectively. This indicated that crude methanol extract of Piper guineense seeds fraction was effective against bacterial pathogens that do cause human disease (Irshad, et al.,.2017; Subramani, et al., 2017).

Similarly, the fractions had inhibitory effects on fungal agents at the least concentration of 3.125mg/ml. The fungi inhibited include Mucor, fragilis, Aspergillus niger, Aspergillius flavus, Candida albicans, Aspergillus fumigatus and Penicillum notatum. This indicated that purifying the seed and extracting the essential oil or ingredient will be helpful as antifungal agent.

The phytochemical, proximate and mineral properties of both the Piper guineense seeds and leaves indicate a range of different diverse biochemical component. These compounds include alkaloids, saponin, flavonoid tannins. These components are very vital to the plants because they protect the Piper guineense plant from microbial invasion and ensure growth of the plant (Uzoekwe and Ezenwajiugo, 2023). These properties were conferred on the methanol extract of the Piper guineense seeds and leaves as they exert inhibitory properties against the bacterial and fungal pathogens investigated. For instance the presence of tannis in the cell of plant was potent inhibitors of many hydrolytic enzymes such as pecteolytic macerating enzymes used by plant pathogens. Tannins have been found to form irreversible complexes with proline rich protein (Shumuda, 2006 and Echo et al 2012] will result in the inhibition of cell protein synthesis herbs that have tannis as their main component are as astringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery. Thus the presence of tannis is a valuable component in killing of bacteria and fungi. Other components such as flavonoids, saponins and alkaloids are also important in folk medicine. Therapeutically, alkaloids are particulary well known as asthetic cardio proletic and anti-inflamatory operation. Well known alkaloids use in clinical setting include morphine, stychine, quinine, ephedrine, nicotine (Hajar, 2015). In this study, Piper guineense had effect against bacterial and fungal pathogens. This may be due to combined effect of alkaloids with other components. It has been reported that alkaloids induces bacterial and fungal synthesis by disrupting bacterial

cell membrane, disrupting inhibiting protein synthesis and affecting DNA formation. It has been suggested that phyto pathogens. Gram negative bacterial are more resistant to alkaloid than gran positive due to an outer hydrophilic, negatively charged layer of lipopolysaccharides

Therefore alkaloids exerts microbial killing due to presence of diverse components such as Quanine and Vinidesine which confer its chemotherapeutic effect (Henrich, et al., 2011). Flavonoids and saponins has more effect on fungal agent have the high susceptibility of fungal pathogens to Piper guineense seeds and leaves, this is because saponins act as permeabilising plasma membrane their amphipathic properties enable them to penetrate membranes where they form complex with sterols and cause pore formation. This pore formation can be on the cell wall of bacterial or fungi thereby releasing the content of bacteria and fungi (Amar et al 1999). The effect of saponins can be reduced due to glycosylation of saponin (Sandrock and Vanetter 1998).Miorrissey and Osbourn 1999). The loss of a single sugar formed the oligosaccharide chain can pair the ability to complex with steroid. Arneson and Durbinm 1967). May fungi can hydrolysed sugar to saponin thereby reducing anti-fungal activity. This effect was seen with the fungal agent Penicillum notatum and Trichophyton ssoudanese where the leaves methanol extract show poor inhibitory effect. The high content of carbohydrate and protein in the methanol extract of Piper guineense seeds and leaves induces balancing effect on the antimicrobial activity of the plant. This is because the carbohydrate and protein act as the nutritional agent to the pathogen which may be antagonistic with impact on the antimicrobial activity. (Ebana, et al.2016)

In folk medicine, Piper guineense seeds and leaves are widely used in various health conditions such as treatment of malaria, respiratory infection and aphrodisc. The leaves in particular are used as a preparation for postpartum woman to encourage uterine involution. The safety of Piper guineense in humans is of importance to protect the individual from any toxic effects. It is normal to test the efficacy of the medicinal plant but in human usage and its domesticated animal. Toxic effect in vivo may impair the function of the cells and tissues of the body system. In this study, the toxic effects of the seeds and leaves of Piper guineense were experimented in an experimental albino rats and its effect on histological kidney and liver biomarkers and tissue damages were investigated. The experimental rats were grouped into five; Group A and B received 500mg/ml and 2000mgt/ml concentration of methanol seeds extracts while Group C and D received 1500mg/ml and 1000mg/ml crude methanol leaves extract respectively while Group E remain normal control that receive normal saline. It was observed that there was increase in weight. The increase in weight of the experimental rats after the experiment may be as a result of the added advantage of the high carbohydrate and protein content of Piper guineense seeds and leaves. This increase in weight was statistically significant which indicates the overall effect of the Piper guineense seeds and leaves and the uptake of the nutritious feeds given to the rats (Nwozo, 2017, Uzoekwe and Ezenwajiugo, 2023)

Hematological indices of toxicity. The seeds and leaves of Piper guineense showed no significant reduction of the hematological parameters though they were slight fluctuation in some parameters. For instance the packed cell volume hemoglobin and total white blood cells indicated slight increases on the estimated values in the test groups when compared with the control group whereas the rate in group B that received the crude metabolic extract of Piper guineense had a decreased in total white cell when compared with the net control (Group E). This decrease might be a chance occurrence in the group. The rats may have been drastically affected by the physiological changes that might have affected the blood volume. Of importance are the platelets in the rates that received seed (Group A) and leaves extract (Group D) that showed significant increase which was higher than the seed extract.

In a similar study Aribo, et al.,(2019) concluded that Piper guineense has little or no hematological effect on experimental albino rats. Therefore the crude methanol extract of Piper guineense seeds and leaves at low concentration may not have effect on blood parameter. It may be suggested that some of the chemical constituent of the extract may have erythropoietic-like effect on the bone marrow leading to the increase in the rate of erythropoiesis' and a resistant increase in packed cell volume and normalizing other indices, (Kolaczynska et al., 1988). The liver plays an active role in the metabolic activities and remains an organ that can be affected by any toxic plant. Therefore, in the study there were elevations of the liver enzyme marker Aspartate transaminase when compared with the control. Aspartate transaminase in the increased group indicated a decrease in estimating values when compared with the net control. There decreases were not statistically significant though it indicates moderate role of the Piper giuneense seeds and leaves. The Alkaline phosphatase also have slight increase of the estimated value of the test group when compared with the control. This indicated that there extract

may have lowering effect of the enzyme alkaline transaminase makers which may or may not protect the liver architecture. It has been shown that alkaline transaminase fluctuates between normal values and elevated value in hepatitis induced liver injury. Thus the toxicity of Piper guineense seeds and leaves extract against the liver may not be detected by the increased liver enzyme markers because the increases did not reflect the level that can be interpreted as having a toxic effect. In a similar study, Mba, et a., (2022) reported fluctuations of enzyme markers especial on alkaline. The experimental rats administered with Piper guineense ethanol extract, the author suggested that Piper guineense may exonerate indicated that this liver enzyme markers may not show liver injure due to ethanol to certain level. Therefore, it can be exploited that Piper guineense methanol extract may have toxic effect if consumed frequently and in high quantity. The electrolytes, sodium, potassium, chloride and bicarbonate and urea concentration were estimated in the serum obtained from the rats.

Healthy functioning of the kidney heart, liver can be accessed using the electrolyte balance in the blood when the level of serum/plasma electrolyte is abnormal; it is believed that the kidney function is impaired. Electrolyte balance can show possibility of the proper maintenance of homeostatic. In this study the concentration of serum electrolytes sodium, potassium, chloride and bicarbonate were not significantly altered in all group administered with crude methanol extract of seeds and leaves of Piper guineense although the sodium, concentration of the sodium and potassium showed fluctuations or increase and decrease among the different groups it can be suggested that Piper guineense seeds and leaves extraction may not adversely interfere with electrolyte in balance thereby suggesting a possible good interaction between the liver and kidney (Imo, et al., 2018, Madueke, et al..,2021) observed that the aqueous extract of Piper guineense seeds has no toxic effect on the kidney in an experimental rats thereby maintain the electrolyte balance in the experimental rats. In this study the feeding of the crude methanol extract of seeds and leaves on the experimental rats do not produce toxic effect on rats therefore making it safe as a spice.

The histological studies of visceral organs showed that the normal (Group E) control had no damage in the visceral organ as expected. The seed extract at a concentration of 500mg/ml body weight showed mild inflammatory cell infiltration in the colon, jejunum, kidney and liver while maintaining relatively body architecture(Nwozo2017). In the rats that received 200mg/ml showed increase in inflammatory cell infiltration within the laminal propria and thickening within the muscular is mucosa while maintaining normal jejunum architecture, normal hepatic architecture and mild to moderate renal tubular cell. All taken together, the seeds had little or no damage to the visceral organs (colon, jejunum, liver and kidney. The bioactive component of the seeds acid may have played a role in protection of the visceral organs because it has been showed to have anti-cancer (colon, prostrate and leukemia) anti-neodegenerative, antiviral (Martens-Talcott et al 2003, Seeram, et al.2006). It has been shown that elegiac acid has the ability to inhibit the growth of pathogen in human (Akiyama, et al.,2001). The non-damaging effect of the crude methanol extract to the visceral organs may have been as a result of biotic agents that are rather protective than toxic. The leaves methanol leaves extract indicated showed no damaging effect of the colon, the jejunum, the liver and the kidney were mildly affected with atrophy of the villi and inflammatory cell. Infiltration on the lamina propria of the jejunum while in the lumen there was focal necrosis and moderate increase infiltration of inflammatory cell and in the kidney. It was observed that there was mild infiltration in the bow man's interstitial cell. The rat in group D that received methanol seed extract 1000mg/ml. maintained relatively normal intact epithelia cells of the cripts of lumberklin. In the jejunum, small intestine, showed normal architecture but there was moderate in the density of inflammatory cells such as lymphocytes within the laminal cells whereas the liver showed the focal necrosis with infiltration on inflammatory cells and the kidney had mild renal dilatation. This indicates that methanol leaves extract of Piper guineense had at higher concentration had toxic effect on the liver and mild changes in the kidney. This change failed to affect the liver function test and electrolytes. This is in agreement with previous studies that showed that there may be liver damage without corresponding increase of the liver biomarkers. The presence of thyzoline has been implicated to have antibacterial, antifungal, anticancer and anti-inflammatory activities, therefore the Piper guineense leaves. With high content of t hyocycline may act as a protective agent against the visceral organs have the mild changes in the organs which can be reversible if the administration of Piper guineense extract and withdrawn nutrient changes may be as a result of the resveratrol. It has been shown that resveratrol had inhibitory effect on H2O2 indicted apoptosis though a prooxidant effect as evidenced by the prominent X-rays in the O2 production which creates a non-conductive intracellular environment for a popotic exercution (Ahmad, et al.2005,) thus the mild damaging effect of the crude methanol extract of the seeds and

leaves may have been created by the presence of resveratrol that is present in both the seeds and leaves. The competitive natures of this bioactive component may have controlled adverse damages of the visceral organs.

CONCLUSION

Piper guineense seeds and leaves possess the following Alkaloids, saponins, soluble carbohydrate, Trepenoids, Phenolic compounds and Saponnins, tannins

Bioactive constituent of Piper guineense (uziza) seed (HPLC) include Epihedrine, Ribalinidine, resveratrol, quinine, Ellagic Acid and kaemferol.

The methanol extract and fractional product of Piper guineense seeds and leaves have both antibacterial and antifungal activities against a wide range of bacteria and fungi.

The seeds and leaves of Piper guineense has shown to be safe but can have toxic effect at higher doses

REFERENCES

1. Abd El-Hack, M.E., El-Shall, N.A., El-Kasrawy, N.I., El-Saadony, M.T., Shafi, M.E., Zabermawi, N.M., Alshilawi, M.S., Alagawany, M., Khafaga , A.F., Bilal, R.M., Elnesr, S.S., Aleya, L., Abuoamar, S.F., El-Tarabily, K.A. (2022). The use of black pepper (Piper guineense) as an ecofriendly antimicrobial agent to fight foodborne microorganisms. Environmental Science Pollution Research International 29(8):10894-10907. doi: 10.1007/s11356-021-17806-7. Epub 2022 Jan 9. PMID: 35000164
2. Abila,B., Richens,A., and Davies,J.A.(1993).Anticonvulsant effects of extracts of the West African black pepper,Piper guineense. Journal of ethnopharmacology, 39(2),113-117
3. Adegbola, J.D.(1972).Molluscicidal properties of some African Plants. Journal of Parasitology;107:108-150.
4. Adegoke, G. O., Gopalakrishna, A. G and Vijayalakshmi, D. (2005). Spices in Food Processing. Journal of Food Science and Technology, 42(4), 311-319.
5. Adesokan, A A., Akanji, MA (2010) Antimalarial bioactivity of Enantia chlorantha stem-bark. Medicinal adverse effects. International Journal of Nutrition Food Sciences 3: 284-289.
6. Agbai,e.o., Onyebuagu, P.C., Njoku,C.J., ,Ekezie,J., Eke, C.C., Nwanegwo C.O., Nwafor, A.C.(2017. Piper guineense leaf extract elevates serum follicle stimulating hormone levej in the diestrus phase in non -pregnant female albino wistar rats. Journal of Complement Alternate Medicine Research,:2:1-8
7. Agonmo,E.N., Onyeike, E.N and Amosike E.O.(2011). The proximate composition and fatty acid profile of Monodora mysticsa(ehuru) and tereptera tereptere(ushokiri.internatioal Research Journal. 3:85-87
8. Ahmad, Al-karthi, A.F.M.., Hena, S.and Khim, L.H(2009.Extraction, Separation and Identification of chemical ingredient of Elepantopus) scarber. Using factoria design of experiment. International journal of chemistry,1:36-49
9. Ahmad,A., Syed,F.A.,Singh S.,,Hadi,S.M.(2005) .Bioxidant activity of resveratrol in the presence of copper ions. Mutagenicity in the plasmid DNA, Toxicologyletticle 159:1-12
10. Akiyama,H., Fujii,K., Yamasaki,O.,Oano,T., Iwutuki,k(2001).Antibacterial action of several tannins against Staphilococcus aureua. Journal of antimicrobial chemotherapy48(4)478-491.
11. Amadi, G., Iwuji, S.C., Azeez, T.O., Nwaokoro, C,J., Wodu, C.O.(2019). Biochemical effect of Piper guineense(African Black pepper) in female dibetics:opportunities for diabetics treatment. International Journal of TranIslated Medicine research. Public Health.3:59-65.
12. Arnesson, P.A., Durbin, R.D.(1967) Hdrolysis of tomatine by Septoria lylopersiciia of detoxification mechanism.Phytopathology.57:1358-1360.
13. Amadi, G., Iwuji, S.C., Azeez, T.O., Nwaokoro, C.J., Wodu, C.O. (2019). Biochemical effects of Piper guineense (African black pepper) in female diabetics: opportunities for diabetes treatment. Internayional Journal Translationl Medical Research Public Health. 3:59–65.
14. Aribo, E.O., Udefa,A.L., Beshel,F.N.(2019) Consumption of Aqueous leaf extract of Piper guineense alters hematological and biochemical parameters inWistar rats..Saudi journal of biomedical research4:3-4

15. Asekun, O. T., Grierson, D. S and Afolayan, A. J. (2006). Antibacterial and antioxidant activities of the volatile oil of Piper guineense. South African Journal of Science, 102(11-12), 580–582.:
16. Ashokkumar, K., Murugan, M., Dhanya, M.. K., Pandian, A., and Warkentin, T. D. (2021). Phytochemistry and therapeutic potential of black pepper [Piper nigrum (L.)] essential oil and piperine: A review. Clinical Phytoscience,7(1), 1-11. https://doi.org/10.1186/s40816-021-00292-2
17. Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F and. Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014
18. Bezerra , D.P., Pessoa, C., de Moraes, M.O., Silveira, E.R., Lima, M.A., Elmiro, F.J., Costa-Lotufo, L.V (2005) Antiproliferative effects of two amides, piperine and piplartine, from Piper species. Naturforsch C Journal of Bioscience. 60:539–543. https://doi.org/10.1515/znc-2005-7-805
19. Bezerra, D.P., Castro FO, Alves, A.P., Pessoa, C., Moraes, M,O., Silveira, E.R., Lima, M.A., Elmiro, F.J., Costa-Lotufo, L.V (2006). In vivo growth inhibition of Sarcoma 180 by piplartine and piperine, two alkaloid amides from Piper. Brazilian Journal of Medical Biology Research 39:801–807. https://doi.org/10.1590/s0100-879x2006000600014
20. Borriss, R., Danchin, A., Harwood, C.R., Médigue, C., Rocha, E.P.C., Sekowska, A., Vallenet, D (2018). Bacillus subtilis, the model Gram-positive bacterium: 20 years of annotation refinement. Microbial Biotechnology 11:3–17. https://doi.org/10.1111/1751-7915.13043
21. Burt, S. (2004) Esential oils, their anti-bacteria properties and potential; Applications in food areview .Internal journal of food Microbiology..94;223-253..
22. Chikezie, P.C., Ojiako, A.O., Nwufo, K.C (2015) Overview of anti-diabetic medicinal plants: The Chrysocoma ciliata L. African Journal of Microbiological Research, 3, 292-297
23. Cheesbrough, M.(2005).District laboratory practice in Tropical countries part1 .2nd edition, Cambridge University Press,New York.pp178-235
24. Dudhatra, G.B., Mody, S.K., Awale, M.M., Patel, H.B., Modi, C.M., Kumar, A., Kamani, D.R., Chauhan, B.N(2012). A comprehensive review on pharmacotherapeutics of herbal bioenhancers. ScientificWorldJournal. 2012;2012:637953. doi: 10.1100/2012/637953. Epub 2012 Sep 17. PMID: 23028251; PMCID: PMC3
25. Ebana,R.U.B., Edet,U.D.,Ekanemesaring U.M., Ikon,G.M., Ekot,G.M. Erok,C.N and Edet,A.P(2016).Antimicrobial activity, phytochemical screening and nutrient analysis of Tetra pleura and Piper guieense. Asian journal of medicine and Health 1:1-8
26. .Echo,I.A., Osuagwu,N.A., Agbor,R.B., Okpako,E.C AND Ekanem, B.E(2012) Phytochemical composition of Aframomum melegueta and Piper guineense seeds. World Joural of AApplied Enviromental Chemistry.2()11:7-21
27. Ekanem, A.P and Obiekezie, A.I.(2000).Antiparasitic effect of leaf extract of Piper guineense (Husani)on the juvenileS of Heterobronchus longifilis (Cuvier and Valencieness). African jourrSnal of fisher Aquaculture. 8,74.
28. Ejele, A. E., Akujobi, C. O and Ogamba, J. O. (2013). Anti-inflammatory and analgesic activities of Piper guineense aqueous extract in rats. Nigerian Journal of Natural Products and Medicine, 17, 11–15.
29. Ene-Obong, H., Onuoha, N., Aburime, L., Mbah O (2018) Chemical composition and antioxidant activities of some indigenous spices consumed in Nigeria. Food Chemistry 238:58–64. https://doi.org/10.1016/j.foodchem.2016.12.072
30. Eseyin, O. A., Ebong, P. E., and Ekpo, A. (2006). Effects of Piper guineense extract on reproductive hormones of male albino rats. Pharmacologyonline, 3, 208–213.
31. Eyob, S., Martinsen, B. K., Tsegaye, Z., & Appelgren, M. (2008). Antioxidant and antimicrobial activities of essential oils from Piper species. Phytotherapy Research, 22(4), 546–549
32. Hajar, R(2015).History of medicine. Time line Hant view.16:43-45.
33. Harvey, A. L., Edrada-Ebel, R., and Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. Nature Reviews Drug Discovery, 14(2), 111–129.
34. Heikens H, Fliers E, Endert E, Ackermans M. Vanmontfrans G, (1995). Liquorice — induced hypertension, a new understanding of an old disease: journal of Medicine, 5:230— 234;1,
35. Heinrich,M., Mah,J., Amirkia,V.(2021). Alkaloids use as medicine:Structural photochemistry meets Biodiversity.An update and forword look.76:1876.
36. Huntington's disease and related neurodegenerative disorders. Journal of Herbal Medicine. 5 1-19.

37. Imo,C., Yakubu, O.E.,Imo,N.G., Udegbunam,I.S., Tatah,S.V., Onukwugha, O.J.(2018). Proxiimate,Mineral and Phytochemical composition of Piper guineense seeds and leaves. Journal of Biological Sciences.18:329-337.
38. Irshad, S., Ashfaq ,A.., Muazzam, A.., Yasmeen ,(2017). Antimicrobial and anti-prostate cancer activity of turmeric (Curcuma longa L.) and black pepper (Piper nigrum 1.) used in typical Pakistani cuisine.Pakistan.Journalof.Zoology.;49:5. doi: 10.17582/journal.pjz/2017.49.5.1665.1669
39. Isaac,, Y..A (2012) characterization and HPLC quantification of piperine in various parts of Piper guineense. Department of pharmaceuticaichemistry, Kwame Nkruma University of Science and Technology, Kumasi.
40. Isikhuemen, E.M., Ogbomwan, B.O., Efenudu, I.U. (2020) Evaluation of phytochemical and mineral constituents of Piper guineense Schum. & Thonn. and Piper Umbellatum Linn: implications for ethnomedicine. European Journal of Medicial Physiology. 31:84–97. https://doi.org/10.9734/ejmp/2020/v31i130209
41. Iweala E.E.J (2015) Anti-cancer and free radical scavenging activity of some Nigerian food plants invitro. International ,Journal of Cancer Research. 11:41–51. https://doi.org/10.3923/ijcr.2015.41.51
42. Iwu M.M (2014). Tradition Medicine in Africa. Indigenous Knowledge and Development ,Monitor, 2(1), 1-12
43. Iwu, M. M. (1993). Handbook of African Medicinal Plants. CRC Press.
44. Iwu, M..M (2014). Handbook of African medicinal plants 2nd edition. CRC Press, Boca Ration Journal of. Pharmacology and phytochemistry., 1(6): 168-182
45. Jabeen, S., Shah ,M.T., Khan, S., Hayat, M. Q. (2010). Determination of major and trace Element in ten important Folk therapeutic plants of HanipurBarn. Journal of medical plant.4(7), 557-566.
46. Javanmerdi ,C.,, Stushnoff, C., Lockie E., Vivanco, M (2003) Antioxidant activity and total phenolic content of Franian Ocimum accession. Journal of food chemistry. 83: 547-50.
47. .Sumathi, P, Parvathi, A. (2010) Aiitimicrobial activity of some traditional medicinal plants. Journal of Medicinal Plant Research, 4, 3 16-321.
48. Kambiré,, D..A.., Yapi,, T..A.., Boti,, J..B.., Garcia,, G.., Tomi, P., Bighelli, A.., Tomi,, F. (2019). Chemical composition of leaf essential oil of Piper umbellatum and aerial part essential oil of Piper guineense from Côte d'Ivoire. National Production Communities. 6:1–8. https://doi.org/10.1177/1934578X19859
49. Kar A (2007) Pharmaocgnosy and; pharmacobiotechnology. 2nd Edition. New Age International Limted Publishres New Delhi: 332-600.
50. Kennedy, D.O., Wightman, E.L (2011) Herbal extracts and phytochemicals: Plant secondary meabo1ites and the enhancement of human brain function. Advance Nutrition 2: 32-50.
51. Kiln Kabán, D. B., Barimala, I..S and Achinewhu, S.C. (2011). Effects of extracts from three indigenous spices on the chemical Stability of smoke dried cat fish (charaslessra). Dip storage. African Journal of food, Agriculture, nutrition and development 11(6:72-85.)
52. Kim, .J, Le ,K.W., Lee, H.J (2011) Cacao (Theobroma cacao) seeds and phytochemicals in human health. In: Nuts and seeds in health and disease prevention. Preedy V, Vatson EE, Patel VB. (Eds.). Academic Press, London, UK: 35 1-60
53. Kolaczynski,J.W.., Ylikahri, R., Harkomen ,M and Koivisto V.A.(1988). Acute effect of ethanol oncounter regulatory response and recovery from insulin induced hypoglycemia. Journal of clinical endoencrinology metabolism. 67(2)384-388.
54. Konczak I, Zhang W (2004) Anthocyanins-More than nature's colours. Journal of Biomedical Biotechnology .: 239-240.
55. Kowaiski. R., Kedzia, B. (2007) Antibacterial activity of Silphium perfoliatum extracts, Pharmaceutical Biology, 45,495-500.
56. Madu ,A..N, Iwu, I..C., Edeh, E.C., Joseph, E..E. (2023), The Extraction and GC-MS Characterization
57. Madueke,S., Ugwu, P.I., Uguru, C.A., Okeke, A.P., Omeire-Oluedo, O.M., Ugwu,S.U., Nwannadi,V.I., Nwachukwu D.C.,(2021) .Continuous intake of high doses of Piper guineense(Ashanti pepper). Aqueous seed extract impairs renal functionin wistar rats. Bioscience,Biotechnology Rsearch Asia.18(3).
58. Manta, S., Saxena ,J., Nema, R., Singh, D and Gupta A (2013). Phytochemistry of medicinal plants. Journal. pharmacogphytochemistry., 1(6): 168-182.

59. Mathur R. (2011) Antimicrobial effect of Phyllanthus ninuri on human pathogenic microorganisms. International Journal of Drug Discovery and Herbal Research, 1, 234-238.
60. Mbongue, F.G., Kantchouing, P., Esame, O.J., Yewah, P.M., Dimo, T., and Lontsi, D. (2005).Effect of the aqeous extract of dry fruits of Piper guineense on the reproductive function of adult male rats.: Indian Journal of pharmacology, 37(1), 30-32.
61. Memudu ,A.E., Akinrinade, I.D., Ogundele, O.M., Dare, B.J (2015) Effects of crude extract of dry fiuiits of Piper guineense on male fertility parameters of adult Sprague Dawley rats. European Journal Medicinal Plant 5: 297-303.
62. Mertens Talcott S, Talcott, S, Perveival, S. (2003) low concentration of quercetina and ellagic acid synergistically influence proliferation, Cytocity and apoptosis in MOLT-4 human leukemia cells. Journal of Brazilian Chemical Soceity 13 (5); 66:610.
63. Mgbeahuruike, E.E , Fyhrquist, P. , Vuorela, H ., Julkunen-Tiitto, R., Holm, Y. (2018) Alkaloid-rich crude extracts, fractions and piperamide alkaloids of Piper guineense posses promising antibacterial effects. Antibiotechnology 7: 98.
64. Mgbeahuruike, E.E., Hoim ,Y., Vuorela, H., Amandikwa, C., Fyhrquist, P. (2019) An ethnobotanical survey and antifungal activity of Piper guineense used for the treatment of fungal infections in West-African traditional medicine. Journal of Ethnopharmacology. 229: 157-166.
65. Mgbeahuruike,, E..E.., Yrjönen,, T.., Vuorela, H., Holm, Y. (2017) Bioactive compounds from medicinal plants: focus on Piper species. South African Journal of Botany 112:54–69. https://doi.org/10.1016/j.sajb.2017.05.007
66. Molluscicidal properties of some African plants. Journal of parasitology 107: 108-15.
67. Mondipa,,F..p., Kamtchoning, P., knoueta, N.,taatchou, J., Fayang, N.P.R., and Mbiapo, F.T. (1999). Effects of aqueous extracts of Hibiscus macranthus and Basellaalba in mature rat testis function. Journal of eythno pharmacology, 65(2), 133-139.
68. Morrissey, J.P., Osbourn, A.E(1999). Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiology of molecule biology review 63:708-724.
69. Naik, A.D., Juvekar, A.R. (2003) Effects of alkaloidal extract of Phyllanthus niruri on HIV replication. Indian Journal of Medical Sciences, 57, 387–393.
70. Negi, J.S., Singh, P., Rawat, B (2011) Chemical constituents and biological importance of swertia: A review. Current Research Chemistry 3: 1-15.
71. Newman, D. J., and Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 1981 to 2019. Journal of Natural Products, 83(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285
72. Ngane, A.N., Biyiti, L., Bouchet, P.H., Nkengfack, A., Zollo, P.A. (2003) Antifungal activity of Piper guineense of Cameroon. Fitoterapia 74:464–468. https://doi.org/10.1016/S0367-326X(03)00112-6
73. Nwachukwu, C.U., Ume, N.C., Obasi, M.N., Nzewuihe, G.U., Onyirioha, C. (2010) The qualitative uses of some medicinal plants in Ikeduru LGA of Imo State, Nigeria. New York Science Journal 3: 132-4.
74. Nwozo, S.O., Ajagbe, A.A., Onyinloye, B.E. (2012) Hepatoprotective effect of Piper guineense aqueous Extract against ethanol induced toxicity in male rats. Journal Experimental Integrated Medicine 2: 71-6.
75. Nwozo, S.O., Lewis, Y.T. and Oyinloye, B.E., 2017. The effects of Piper guineense versus Sesamum indicum aqueous extracts on lipid metabolism and antioxidants in hypercholesterolemic rats. Iranian Journal of Medical Sciences, 42(5), p.449.
76. Obadina, A.O and Ogundumu A.A Microbial Contamination of Selected dietary Supplements in a typical Market in Nigeria. Nigeria food Journal, 2011.2 (4) 100-102.
77. Ogunniran, K.O (2009). Antibacterial effects of extracts of Ocimum gratissimum and Piper guineense on Escherichia coli andStaphylococcus aureus. African Journal of Food Science. 3:77–81
78. Ogunmefun,T.O., Akharaiyi,F.C and Adegunle,S.J.(2017).Phytoghemical andAntimicrobial properties of Piper guineense (Shumach and Thonn) on selected humanpathogens. Journal of chemical and Pharmacetical Research,9(11):180-186.
79. Ohiagu, F.O., Chikezie, P.C., Maduka, T.D.O., Enyoh, C.E., Chikezie, C.M.(2021) Bioactive compound and medicinal usefulness of edible leaves of Vernonia amygdalina, Ocimum gratissimum, Piper guineense and Gongronema latifolium. SAJ Pharmaceutical Pharmacology 7: 101).

80. .Ojinnala, M.C.,, Odiegwu, E.N., and Chichebere, F.E. (2016). Comparative Study on the nutrient and anti-nutrient composition of the seeds and Leaves of Uziza (Piper guineemse) Journal of Environmental science,Toxicology and food technology, 10(8),42-48.
81. Okeke, M.I., Iroegbu, C.U., Jideofor, C.O., Okoli, A.S., Esimone, C.O (2001) Anti-microbial activity of ethanol extracts of two indigenous Nigerian spices. Journal Herbs Spices of Medicinal Plants 8:39–46. https://doi.org/10.1300/J044v08n04_
82. Okokon, J. E., Umoh, E. E., and Essien, E. E. (2012). Antiplasmodial activity of Piper guineense fruit extract. Indian Journal of Pharmacology, 44(3), 384–387.
83. Okolo, S.C., Okoh-Esene, R.U., Ikokoh, P.P., Olajide, O.O., Anjorin, S.T. (2012) Phytochemicals, mineral content and antimicrobial screening of Phyllanthus amarus Schum and Thonn in Abuja, Nigeria Journal of Microbiology and Biotechnology Research, 2, 17-22.
84. Okon,, E ..E., Egbuna, C., Odo, C..E.,., Nsikan, M., Awah, F.M (2013) In vitro antioxidant and nitric oxide scavenging activities of Piper guineense seeds. Journal of Ethnopharmacology 2: 4854-94.
85. Okonkwo, J. O., Eme, P. E., and Anekwe, G. E. (2016). Phytochemical and antimicrobial activities of Piper guineense. African Journal of Plant Science, 10(9), 181–186.
86. Okoye, E.I and Ebeledike, A.O (2013). Phytochemical constituents of Piper guineense (uziza) and their health implications on some microorganisms. Global Research. Journal of Sciences.2 (2): 42-46
87. Okwu, D.E (2001). Evaluation of the chemical composition of indigenous species and flavoring agents. Global Journal of. Pure and Applied . Sciences.. 7;455-459.
88. Oma,r H.H., Shiekh, H.M., Gumgumjee, N.N., El-Kazon, M.M., El-Gendy, A.M. (2012) Antibacterial activity of extracts of marine algae from the Red Sea of Jeddah, Saudi Arabia. African Journal of Biotechnology, 11, 13576-13585.
89. Omodamiro, O.D., Ekeleme, C.M (2013) Comparative study of in vitro antioxidant and antimicrobial activities of Piper guineense, Curmuma longa, Gongronema latifolium, Allium sativum, Ocimum gratissimum. World Journal of Medical Sciences 1: 51-69.
90. Oyemitan, I.A., Olayera, O.A., Alabi, A., Abass, L.A, Elusiyan, C.A., Oyedeji, A.O., Akanmu, M.A. (2015) Psychoneuropharmacological activities and chemical composition of essential oil of fresh fruits of Piper guineense (Piperaceae) in mice. Journal of Ethnopharmacology 166:240–249. https://doi.org/10.1016/j.jep.2015.03.004
91. Paithankar, V.V., Raul, K.S., Charde, R.M., Vyas, J.V. (2011) Phyllanthus ninuri: A magic herbs. Research in Pharmacy, 1, 1-9.
92. Pa,,D and Verma,P (2013). Flavonoids: A powerful and abundant source of antioxidants. International. Journal of Pharmaceutical. Sciences. 5(3): 97.
93. Ramawat, K.G., Dass, S., Mathur, M. (2009) The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Herbal Drugs: Ethnomedicine to Modern Medicine, Ramawat KG. (Ed.). Springer, New York: 7-32.
94. Rates, S. M. K. (2001). Plants as source of drugs. Toxicon, 39(5), 603–613. https://doi.org/10.1016/S0041-0101(00)00154
95. Rodriguez-Ramiro, I., Ramos, S., Lopez-Oliva, E., Agis-Torres, A., Bravo, L., et al. (2013) Cocoa polyphenols prevent inflammation in the colon of azoxymethane-treated rats and in TNFct-stimulated Caco-2 cells. British Journal of Nutrition 110: 206-215.
96. Sandrock,R.W., Vanetten, H.D(1998) Fnugal Sensitivity to tomatinase activity in phytoanticipin and tomatine. Phytopathology 88:137-143.
97. Scott, I.M, Puniani, E., Jensen, H., Livesey, J.F., Poveda, L., Sanchez-Vindas, P., Durst, T., Arnason, J.T (2005) Analysis of piperaceae germplasm by HPLC and GC-MS: a method for and Food Chemistry isolating and identifying unsaturated amides from Piper species extracts. Journal of Agriculture 53:1907–1913. https://doi.org/10.1021/jf048305a
98. Seeram,N.P.,Adams, L.S., Zhang, y., Lee,R.,Sand,D.,Scheuller, H.S.,Heber,D.(2006).Blackberry, blackraspberry ,blueberry, cranberry, redraspberry, and strawberry extract inhibit growth andstimulate apoptosis of humancancer cells in vitro.Agricultural of food and chemistry.54(25):9329-9339.
99. Selvamohan, T., Ramadas, V., Kishore S.S.S.. (2012) Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advances in Applied Science Research, 3, 3374-3381.

100. Sharma P, Parmar J, Verma P, Sharma P, Goyal PK. (2009) Anti-tumour activity of Phyllanthus ninuri (medicinal plant) on clinical-induced skin carcinogenesis in mice. Asian Pacific Journal of Cancer Prevention, 10, 1089-1094
101. Sharma, A., Shanker, C., Tyagi, L. K., Singh, M., & Rao, C. V. (2019). Herbal medicine for market potential in India: An overview. Academic Journal of Plant Sciences, 2(2), 26–36.
102. Shimanda,T(2006).Phytochemical,Antimicrobial properties of Piper guineense on selected Human pathogens. Journal of chemical Ecology 32(6)1149-1163.
103. SubramanI, R., Narayanasamy, M., Feussner, K.(2017) Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. Biotechnology. 7:172. doi: 10.1007/s13205-0o17-0848-9
104. Sumathi, P., Parvathi, A. (2010) Antimicrobial activity of some traditional medicinal plants. Journal of Medicinal Plant Research, 4, 316-321.
105. Tankam, J.M., Ito, M. (2013) Inhalation of the essential oil of Piper guineense from Cameroon shows sedative and anxiolytic-like effects in mice. Biology Pharmacology Bulletin 36: 1608-1614.
106. Tekwu, E.M., Askun, T., Kuete, V., Nkengfack, A.E., Nyasse, B., Etoa, F., Beng, V.P(2012). Antibacterial activity of selected Cameroonian dietary spices ethno-medically used against strains of Mycobacterium tuberculosis Journal of Ethnopharmacology, 142: 374.
107. Tomsone, L., Kruma., Galoburda, R. (2012) Screening of Phyllanthus species for antimicrobial properties. Chemical Sciences Journal, 56.
108. Udoh, F.V. (1999). Uterine muscle reactivity to repeated administration and phytochemistry of the leaf and seed extracts of Piper guineense. Phyto-therapy Research: An international Journal Devoted to pharmacological and Toxicological Evaluation of Natural product Derivatives, 13(1), 55-58
109. Uzokwe,N.M. and Ezenwanyiugo,C.E(2023).Phytochemical,Elemental and Proximate analysis of Piper guineense leaves. Journal of Applied Science and Environmental management.27(4)657-663.
110. Udoh, F.V., Akpan, ,J.O and Ufuruma,N.(1996).Effect of extract of leaves and seeds of Piper guineense of some smooth muscle activity in rat,guinea pig and rabit. Phytotherapy research. 10(7),596-599.
111. Udoh, F.V., Lot, T.Y., Brocide, V. B. (1999). Effects of Extracts of seeds and Leaf of Piper guineense on skeletal museles Activity in Rat and frog. Phytotherapy Research 13:106-109
112. Udoudoakpan, I.F and Effiong B.N.(2024).Accessing the Antimicrobial potency of Piper guineense(Odusa) seed extracts on bread spoilage organisms.Research journal of foodscience and quality control. 10:5..
113. Uzokwe, N.M, Ezenwayiugo, C.E. (2023). Phytocemicals, Elemental and Proximate analyses of Piper guineense leaves Journal of Applied Science and Environmental Mangement.27(4)657-663.
114. Velayutham, R., Sankaradoss, N., Ahamed, K.N (2012) Protective effect of tannins from Ficus racemosa in hypercholesterolemia and diabetes induced vascular tissue damage in rats. Asian Pacific Journal of Tropical Medicine. 5: 367-373.
115. World Health Organization (2014) antimicrobial Resistance Global Report on Surveillance. World health organization genera, Switzerland: VB. (Eds.). Academic Press, London, UK: 351-60.
116. World Health Organization (2014) antimicrobial Resistance Global Report on Surveillance. World health organization geneva, Switzerland:
117. World Health Organization (WHO) (2006) International cardiovascular disease statistics 7-8
118. Yokozawa, J.,, Cho, E.J., Park, C.H., Kim, J.H. (2011) Protective effect of proanthocyanidin against diabetic oxidative stress. Evid Based Complement Alternative Medicine 2011: 623879.