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# TOXICITY OF CRUDE METHANOL EXTRACT OF PIPER GUINEENSE (UZIZA) SEEDS AND LEAVES

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## ABSTRACT

Due to traditional use of *Piper guineense* leaves and seeds, it is of relevance to examine the efficacy of *Piper guineense* leaves and seeds against drug resistant strains of some selected clinical bacteria and fungi. Some studies have verified the nutritional benefits and antimicrobial activity of *Piper guineeense* but there is little or no information on its possible toxic effects. It is a known fact that any substance with antimicrobial activity could have potential for toxicity. Phytomedicine is becoming increasingly popular with little regard to the possible toxic

. *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 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

## II. INTRODUCTION

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.

### 1Taxonomic Classification

*Piper guineense*, commonly referred to as West African black pepper or Ashanti pepper, belongs to the family **Piperaceae**, a family well known for its aromatic and medicinally valuable members. This species is indigenous to the tropical regions of West Africa and is widely used for culinary and ethnomedicinal purposes.

The classification of *Piper guineense* is as follows:

- **Kingdom:** Plantae
- **phylum:** tracheophyta

- **Class:** Magnoliopsida
- **Order:** Piperales
- **Family:** Piperaceae
- **Genus:** *Piper*
- **Species:** *Piper guineense* (Schumach. & Thonn).

This species was first described by Heinrich Christian Friedrich Schumacher and Peter Thonning in the early 19th century (Schumacher and Thonning, 1827).

The *Piperaceae* family is predominantly tropical, comprising more than 1,000 species. Members of this family, including *P. guineense*, are known for their essential oils and secondary metabolites, which contribute to their roles in traditional medicine and as natural pesticides (Burt, 2004).

In African ethnobotany, *Piper guineense* is significant not only for its pungent flavor but also for its antimicrobial and anti-inflammatory properties, which align with the traits found within the Piperaceae family (Mgbeahuruike, et al., 2019).

*Piper guineense* is closely related to other members of the *Piper* genus, such as:

- *Piper nigrum* (black pepper)
- *Piper betle* (betel leaf)
- *Piper methysticum* (kava)

While it shares morphological features such as climbing stems, ovate leaves, and spiked inflorescences (Okwu and Morah, 2004) and pungency with *Piper nigrum*, *Piper guineense* is genetically distinct and adapted to African climates and soil conditions (Eyob, *et al.,* 2008).

## II. 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).

### Toxicity of Crude Methanol Extract in Experimental Rats

A total of thirty (30) albino Wistar rats were used to check the safety of *Piper guineense* leaves and seeds. The rats were purchased from the animal house at the faculty of Veterinary medicine, University of Nigeria Nsukka (UNN) and transported to the Department of Anatomy, University of Nigeria Enugu Campus (UNEC). The rats were allowed to acclimatize for 14days. They were weighed and grouped according to their nearest weight into five (5) groups (A-E). The rats in group A and B received 500mg and 200mg of crude methanol extract of *Piper guineense* seeds respectively while the rats in group C and D received 1500mg and 1000mg of the crude methanol extract of *Piper guineense* leaves respectively. The rats in group E were given normal saline only. All the rats were fed with water and feed ab initio. The humane handling of animals was totally adhered to. An ethical approval was obtained on the ethical handling of animal at the Ministry of agriculture and agro industrialization, veterinary services Department, Enugu state.. The rats were administered with the extract via oral route for fourteen (14) days and observed for behavioural charges. At the termination of the experiment on the 14$^{th}$ day, the rats were fasted and on the fifteenth (15th) day, the rats were reweighed. Blood samples were collected via the orbital vein into EDTA bottles and Plain bottles. The blood in the plain bottle was allowed to clot and serum collected and stored at – 20$^{o}$C until used. The blood collected in EDTA bottles was transported to the lab and analyzed immediately for hematological parameters.

The animals were scarified using cotton wool soaked chloroform in a small plastic can. They were dissected and the liver, kidney, and intestine were collected and preserved in 10% formal saline for histological study.

**Table 1.1: Study Protocol**

| Group | Rats | Treatment | Concentration (mg/ml) |
|---|---|---|---|
| A Seed | 6 | Seeds | 500 |
| B Seed | 6 | Seeds | 200 |
| C Leaves | 6 | Leaves | 1500 |
| D Leaves | 6 | Leaves | 1000 |
| E Normal control | 6 | Control | Normal saline |

**. 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 ÷ $20\mu l$ $(0.02ml)$ 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 100$^{o}$C for 15 minutes on a heat –block set at 100$^{o}$C 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

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

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.

## III. 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).

**Antibacterial activity of crude methanol extract of *Piper guineense.***

**.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 guineense*was 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.

**: Toxicity of Crude Methanol Extract of *Piper guineense* using experimental albino Rats.**

A total of Thirty (30) albino rats were used to check for the toxicity of the seeds and leaves of *Piper guineense.* A predetermined concentrations were administered to the rats (Table 3.2). At the end of the experiment, the weights of the animals both before and after administration differed as there was increase in weight in group A, B, C, D and control E (seeds). The increment in weight was statistical significant in Group A, Group D and Group E.

**Table 1.2: 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 1.3: 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 1.4: 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)

**Table1.7: shows toxicity of the Crude Methanol extract on biomarkers of the liver in an experimental albino Rats**

The liver biomarkers, Total Bilirubin(TBL). *Aspartate Trainsairnase*(AST) *Alanine iTrausaminase*(ALS) were estimated. The AST showed that the control group with 54.8±405M/L was statistically significant when compared with group C (Leaves extract) with 65.24±3.26µ/l was statistically significant when compared with group C (leaves extract) with 65.24±3.26µ/l. P= 0.037. Other estimated parameters of Total Bilirubin, Alanine Transaminase were not statistical significant on both the leaves and seeds methanol administration.

**Table 1.6: Toxicity of *Piper guineese* methanol extracts on blood parameters**

| Group | PCV(%) | Hb | RBC | TWBC | GRA | LYM | MID | PLT |
|---|---|---|---|---|---|---|---|---|

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A Seeds | 53±2.86 | 176.25±9.71 | 7.44±0.48 | 7.44±2.05 | 9.13±2.58 | 85.53±2.33 | 5.35±0.82 | 22.5±91.85 |
| B Seeds | 46.38±10.0 | 154.25±33.94 | 6.11±1.36 | 3.69±0.92* | 9.33±3.83 | 86.98±4.89 | 3.7±1.06 | 189.5±189.04 |
| C Leaves | 59.78±6.11 | 194±14.07 | 8.33±0.40 | 5.92±2.29* | 5.38±1.33 | 90.60±1.15 | 4.03±0.40 | 195.5±57.28 |
| D Leaves | 53.63±2.38 | 177.75±8.66 | 6.62±0.19 | 5.81±0.99* | 5.0±0.79 | 91.48±1.19 | 3.73±0.57 | 202.525±61.99 |
| E Control | 47.80±12.16 | 158.5±3.92 | 5.20±1.34 | 6.26±1.74 | 5.55±1.89 | 90.5±1.39 | 3.95±0.78 | 106.0±67.60 |

**Table 1.7: Toxicity of crude methanol extract of *Piper guineese* on the liver of experimental rats.**

| GROUPS | TOTAL BILIRUBIN (mg/dl) | TRANSANNINASE (u/l) | TRANSAMINASE (u/l) | Alkaline phosphatase (U/L) |
|---|---|---|---|---|
| Group A Seed extract | 0.58±0.05 | 64.4±4.5 | 29.01±2.0 | 131.73±3.34 |
| Group B seed extract | 0.44±0.11 | 59.33±3.97 | 30.5±1.21 | 81.18±4.22 |
| Group C leaf extract | 0.46±0.06 | 65.24±3.26 | 30.43±2.55 | 90.08±1.12 |
| Group D leaf extract | 0.39±009 | 58.0±2.10 | 33.46±1.45 | 83.49±3.43 |
| Group E control | 0.30±0.04 | 54.8±4.05 | 33.29±3.33 | 75.43±3.06 |



**Estimation of electrolyte and urea levels**

The electrolytes, sodium, Potassium Chloride and bicarbonates were not statistically significant when compared with the control (Group E). The estimated value of potassium indicates that control was 5.7±1.05 while in group A,B,C and D the value were 5.4±1.31, 5.5±1.20, 6.1+1.17 5.3±1.9mmol/L respectively which did not differ when compared with control. The urea was elevated in group C 9.15±1.21 that received the leaves extract at concentration of 1500mg/ml and 1000mg/ml when compared with the control group E.(Table 4.12)

**Table 1.2: Phytochemical, Proximate and mineral content of methanol extract of *Piper guineense* seeds and leaves.**

**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, 5.80±0.05 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..

## IV. 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 P*iper 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.

## V. 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

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