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BIOACTIVE (GCMS) COMPONENTS AND FUNGAL ACTIVITY OF CRUDE METHANOL EXTRACT OF PIPER GUINEENSE (UZIZA) SEEDS AND LEAVES

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ABSTRACT

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 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.

1.0 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).

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.

. 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; S*taphylococcus aereus* and Streptococeus *pyogenes*.

TABLE1.0: Sources of microbial isolates:

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

Method

AST – (Colorimetric method).

Assay Principle

The reaction involved in the assay system is as follows:

The amino group is enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate.

L-Aspartate AST Oxaloacetate

\+ ⟶ +

2-Oxoglutarate L-Glutamate

AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine

Procedure

1. Measurement against Reagent Blank

Pipette into test tubes

	Reagent blank	Sample
R1(buffer)	0.5 ml	0.5 ml
Sample	------	100 µl
Distilled water	100 µl	------
Mix and incubate for exactly 30 minutes at 37 ºC		
R2	0.5 ml	0.5 ml
Mix and incubate for exactly 20 minutes at 20 – 25 ºC		
Sodium hydroxide	5.0 ml	5.0 ml

Mix and measure absorbance of specimen against reagent blank at 546 nm after 5 minutes.

2. Measurement against Sample Blank

	Sample blank	Sample
R1(buffer)	0.5 ml	0.5 ml
Sample	-----	100 µl

Mix and incubate for exactly 30 minutes at 37 ºC

R2	0.5 ml	0.5 ml
Sample	100 µl	-----

Mix and incubate for exactly 20 minutes. at 20 – 25 ºC

Sodium hydroxide	5.0 ml	5.0 ml

Mix and measure absorbance of specimen against sample blank at 546 nm after 5 minutes.

Alkaline Phosphatase

Alkaline phosphatase (ALP) catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium (pH optimum 10). The liver and biliary tract are the source of alkaline phosphatase in normal sera. Normal alkaline phosphatase levels are age dependent being higher in children and adolescents in comparison to adults. ALP is one of the tests of choice for evaluating cholestasis and obstructive juandice. Elevated levels are found in many diseases including hepatitis, cirrhosis, malignancy, and in bone diseases.

Method

ALP – (Colorimetric method).

3.6.6.3.2: Assay Principle

Colorimetric determination of alkaline Phosphatase activity according to the following reaction:

Phenylphosphate $\xrightarrow[pH\ 10]{ALP}$ Phenol + Phosphate

Phenol liberated is measured in the presence of 4-aminoantipyrine and Potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Procedure

Set up the following tubes

	Serum Sample	Serum blank	Standard	Reagent blank
R1	2 ml	2ml	2ml	2ml

Incubate for 5 minutes at 37 ºC

R2	.....	.....	50 µl	.....
Serum	50 µl	.....	.....	.....

Incubate for exactly 15 minutes at 37 ºC

R3	0.5 ml	0.5 ml	0.5 ml	0.5 ml

Mix well or preferably vortex.

R4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	.....	50 µl	.....	.....
Distill .Water	.....	.....	.....	50 µl

Mix, let stand for 10 minutes in the dark then measure.

The color intensity is stable for 45 minutes.

Calculation

$$\frac{\text{OD serum sample} - \text{OD serum blank}}{\text{OD Standard}} \times n$$

n = 20 (Kind and king U/100 ml)

n = 142 (IU/L)

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.*

.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 1.2)

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(Table 1 3)

TABLE1.1: 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)
Aspergillus fumigatus	Sputum
Penicillium notatum	Hand
Trichophyton souduense	Foot nail Scrapping
Candida albicans	High vaginal swap

Table 1.2. 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 1.3: 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 -										

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.(Table1.4)

.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. 5)

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 4.6)

Table 1.4: 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 1.5: 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	-	-	-	-	-	-	+	+	+
Mucor fragilis	-	-	-	-	-	+	+	+	+
Candida albicans	-	-	-	-	-	+	+	+	+
Aspergillu sfumigatus	-	-	-	-	+	+	+	+	+
Trichophyton soudanense	-	-	-	-	+	+	+	+	+
Penicillium notatum	-	-	-	+	+	+	+	+	+

Table 1.6: 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 aeroginosa	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 4.7)

. 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 4.8)

: 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.(Table1.6)

Bioactive Components of *Piper guineense* seeds and leaves.

Different bioactive components were detected using High Performance liquid chromatograph (HPLC). The leaves had resveratrol at a concentration of 39.24ug/ml while anthocyanidines, flavonones, proanthocyanidines were detected at a concentration of 20.23 and 85.48µl/ml respectively .The seeds have different bioactive component different from the leaves; Epihedrine, rutin, ribalniidine, and ellagic acid. Quinine was detected at different concentration 60.06 and 44.03µl/ml. The other components include Kaemferol; 4.25µl/ml; rutin 12.04µl/ml, and Resveratol 10.90µl/ml.(Table 4.17)

Table 1.8:: 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		
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

Table1.9: 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.(Fig1.2)

GROUP A

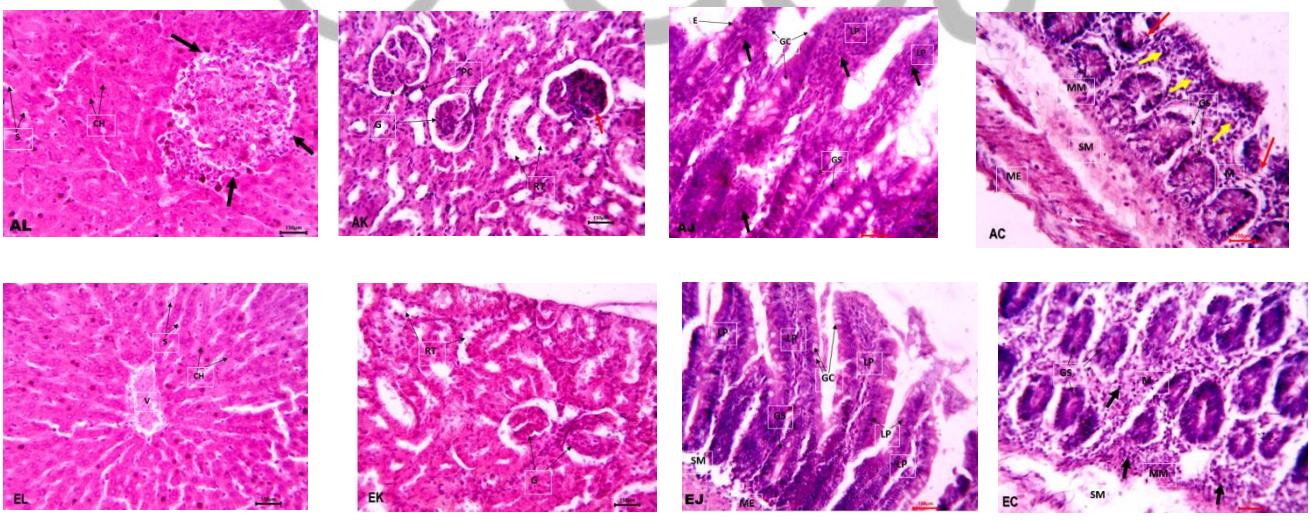

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Figure: .1.1… Photomicrographs Comparing Histological Changes in Liver, Kidney, Jejunum, and Colon Tissues of Albino Rats in Experimental Group A and Normal Control

GROUP B

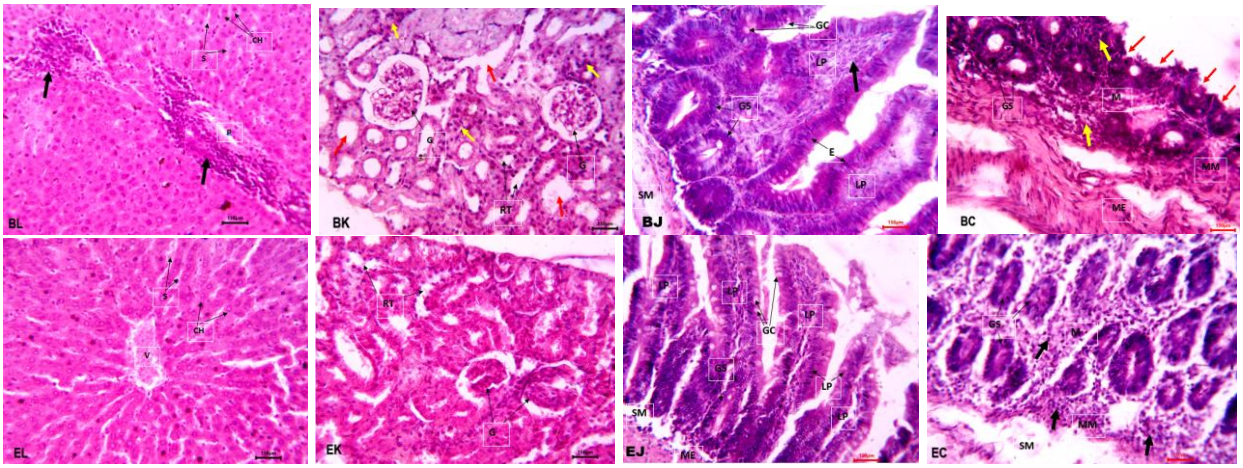

Figure 1.2….: Photomicrographs Comparing Histological Changes in Liver, Kidney, Jejunum, and Colon Tissues of Albino Rats in Experimental Group B and Normal Control

GROUP C

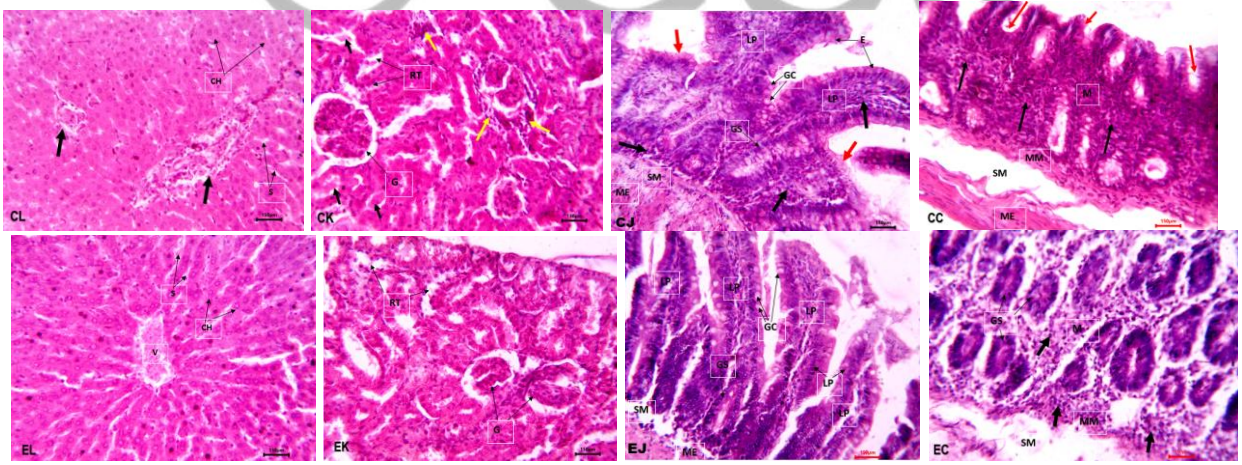

Figure 1. 3…: Photomicrographs Comparing Histological Changes in Liver, Kidney, Jejunum, and Colon Tissues of Albino Rats in Experimental Group C and Normal Control

GROUP D

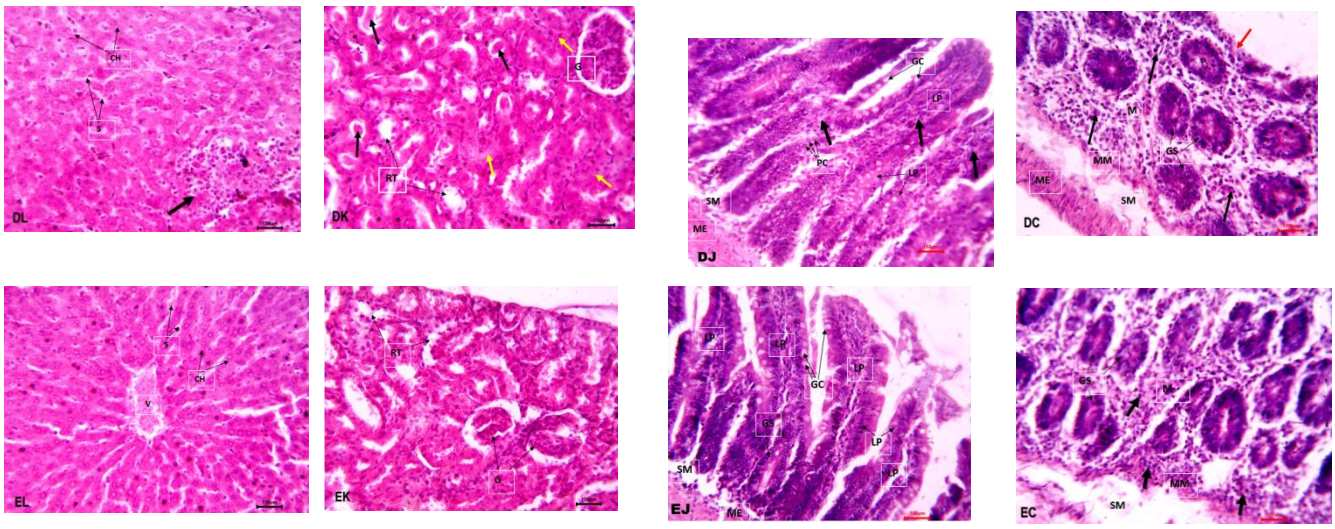

Figure 1 .4…. : Photomicrographs of the liver, kidney, jejunum, and colon of albino rats showing histological alterations in the experimental group compared to the normal control group.

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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