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NUTRITIONAL, MINERAL CONTENT, PHYTOCHEMICAL, ANTIOXIDANT EVALUATION, AND ANTIMICROBIAL SCREENING OF *Pterocarpus Osun* LEAF EXTRACT.

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ABSTRACT

Pterocarpus osun is an evergreen plant, found in many tropical areas of the globe. The plant parts have been adopted for traditional therapy of many diseases, like sickle cell disorder. This study is to determine the phytochemical, antimicrobial, and nutritional constituents of the leaf extract. The leaf was investigated for its nutritional, mineral properties, antioxidant activity, and biological properties. The powdered leaf was extracted with dichloromethane, ethyl acetate, and methanol successively. Phytochemical analysis of the extracts shows that they contain alkaloids, flavonoids, and terpenoids in dichloromethane extract. Tannins, alkaloids, flavonoids, and terpenoids were present in ethyl acetate and methanol extract. The extracts were tested against *staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella sp,* and microorganisms. The mineral composition showed that it contained Ca: Mg: Fe: Mn: K: Na: and Cu: The radical scavenging activities revealed at 0.125mg/ml, the % inhibitory concentration of ethyl acetate extract showed the highest 74.97% as compared with 75.93% vitamin C standard. The investigation revealed the presence of alkaloids, flavonoids, and tannins in all. It revealed the highest quantity of calcium in the leaf extract, the presence of minerals in the leaf can ensure adequate removal of antinutritional.

Keywords: *Pterocarpus osun*, Proximate, Alkaloids, *Escherichia coli*, Antioxidant.

INTRODUCTION

Pterocarpus Osun Craib is an evergreen tree with a spreading crown, growing 12-30 meters tall. *Pterocarpus osun* are found generally in many tropical areas of the globe, especially in Africa and Asia, where they are used for the management of a wide range of ailments [1]. The plant belongs to the family of *Fabaceae*, the common names in English are Canwood and Blackwood. In the Yoruba language it is called Osun–dudu, Gbingbin, and in the Igbo language, it is called Akwara. The plant *Pterocarpus osun* has been found to have enormous application from leaves to roots and its ethno-pharmacology properties.

Pterocarpus species is an endangered species as a result of human activities [2]. It is one of such plants that have been used for the treatment of type 2 diabetes [3]. The species have also been used in treating diarrhea and wound powder has been externally applied in the treatment of inflammations, headaches, mental aberrations and ulcer [4]. The species has also confirmed the presence of various

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components, such as carbohydrates, steroids, anthocyanins, saponins, tannins, phenols, triterpenoids, flavonoids and glycosides [5].

The plant parts have been adopted for traditional therapy of many diseases, like sickle-cell disorder and amenorrhea [6]. The dry leaf is used for blood supplements and is also an ingredient of traditional black soap that is based on ash burnt cocoa pod and palm oil. In the Western region (Yoruba) of Nigeria, *Pterocarpus Osun* is used in various health conditions including sickle-cell anemia [7]. These species are also known to be of Agricultural importance as they co-fertilize on the soil via Nitrogen-fixing bacteria in their nodules

MATERIALS AND METHODS

Sample Collection and Preparation

The plant leaves were collected from the Sheda Science and Technology Complex (SHESTCO) in Sheda near Kwali, Abuja. The collected materials were air-dried at room temperature for two weeks. The sample leaves were crushed using 240V 4L blender (Thomas Scientific, Swedesborn, UK). The Crushed sample was stored in an air-tight bag assay for analysis.

Reagents

Most of the chemicals used were of the analytical grade, deionzed and distilled water were used throughout the experiment.

Preparation of extracts

The powdered leaf sample was extracted using the cold method (Cold Maceration). The solvents used were n-hexane, dichloromethane, ethyl acetate, and methanol successively for a period of eight to twelve hours each. At the end of the period, the solvent was recovered by rotary evaporator. The extracts were then transferred to desiccators and allowed to cool before it was analyzed. The low temperature employed will help preserve the nature and quality of temperature-sensitive components of the plant tissues [8].

Phytochemical Screening

The phytochemical screening of the leaf extract was conducted in the Chemistry Advanced Research Center (CARC), SHESTCO, Sheda, Abuja. The extract was analyzed for the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and cardiac glycosides in line with the procedure described[9,10].

Proximate Analysis

The ash and moisture contents were determined as described by AOAC [11]. The crude fat was extracted by the Soxhlet method with petroleum ether 40^0C-80^0C for eight hours (8hrs). The total Nitrogen was determined using the micro Kjeldahl method and converted to crude protein content by multipling with 6.25. The carbohydrate content was determined by the

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percentage difference of the various others. The determinations were done in triplicate and results were expressed as the average of percentage values on dry weight basis [12].

Determination of Minerals Content

The mineral content of the sample was analyzed by the AOAC method [11,13], using an Atomic Absorption Spectrophotometer. The digested samples in Conc.HNO_3 was made up to 100 ml and stored in a polypropylene container. Absorbance was noted for the standard solution of each element in the samples, using Atomic Absorption Spectrophotometer (AAS).

Determination of Anti-oxidant activity

The radical scavenging activity of the plant extract against 2, 2-Diphenyl-1-picrylhydrazyl radical (Sigma-Aldrich) was determined by UV-Spectrophotometer at 517nm. The radical slightly modified method previously described [14,15]. The following concentrations of the extracts and standard (Vitamin C) were prepared; 0.5, 0.25, 0.125, 0.0625, and 0.0312mg/ml in methanol (Analar Grade). 1ml of the extract was placed in a test tube and 3ml of 1mMDPPH in methanol.

A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = (A_b - A_a / A_b) \times 100 \text{ ---------[1]}$$

Where A_b is the absorbance of the blank Sample (without the extract)

A_a is the absorbance of the extract

Antimicrobial Screening

The antimicrobial screening of the leaf extract was carried out by the well diffusion method [15]. The plates were prepared by pouring sterile Muller Hinton agar into sterile petri dishes that were autoclaved. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates. Wells of equal size were cut with proper gap in the medium and the extracts were added into it. The plates were allowed to stand for one hour, to allow pre-diffusion of the extract into the medium [16]. The plates were incubated at 37^0C for 24 hours. The standard drugs used were Sparfloxacin, Ciprofloxacin and Streptomycin. At the end of the incubation period, inhibition zone was measured in millimeter. The study was carried out in triplicate.

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemicals are present in dichloromethane, ethyl acetate, methanol, aqueous, and ethanol extract of *pterocarpus Osun* leaf as shown in Table-1. The investigation also revealed the presence of alkaloids, flavonoids and tannins in all the solvent system used for extraction, which suggest that it may be used as

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basic natural ingredient for antifungal and antimicrobial agents. Terpenoids is present except in ethanol and aqueous leaf extract. Saponins were present in aqueous and ethanol extract but absent in dichloromethane, ethyl acetate and methanol extract. The presence of saponins may help in reducing the burden of the liver as regards cholesterol metabolism [17]. Flavonoids was reported to act as antioxidant, inhibitors of cyclo-oxgenate and 5- lipoxgenase such that additional anti-inflammatory and thrombolytic aggregation inhibitory effect can be expected [18].Cardiac glycosides was present in dichloromethane, methanol and ethanol but absent in ethyl acetate and aqueous extract of the leaf. Cardiac glycosides help improve cardiac output in people with heart failure and also slow down heartbeats that are too fast. [19]. It has been reported that plants occur in varying habitats has a great magnitude of variation in the concentration and composition of phytochemical ingredients in the different part of such plants is expected [20]

Table 1: PHYTOCHEMICAL SCREENING

No.	Parameters	Dichloromethane	Ethyl acetate	Methanol	Aqueous	Ethanol
1	Alkaloids	+	+	+	+	+
2	Flavonoids	+	+	+	+	+
3	Terpenoids	+	+	+	-	+
4	Phenol	-	+	+	+	-
5	Saponins	-	-	-	+	+
6	Tannins	+	+	+	+	+
7	Cardiac glycoside	+	-	+	-	+

Proximate analysis

Table-2 Investigates the proximate analysis of the leaf as having high carbohydrate value of 25.55%, followed by fibre content 32.33% which is closely followed by lipid content 13.43% with protein value 12.24% while the ash content 8.95% shows reasonable low moisture content of 7.50%.

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Table 2: PROXIMATE ANALYSIS

Parameters	Moisture	Lipid	Ash	Fibre	Proteins	Carbohydrate
Percentage (%)	7.50	13.43	8.95	32.33	12.24	25.55

Antioxidant activities

Table-3, revealed at 0.5mg/ml concentration the reducing order of inhibitory activity of the leaf extracts compared with vitamin C standard (75.93%): FILT/DCM/POS(50.75%)>MTH/POS(50.53%).

At 0.25mg/ml conc. MTH/POS (57.67%)>ETOA/POS (52.19%) compared with vitamin C 75.13%. At 0.125mg/ml conc. Showed ethyl acetate extract as the highest inhibitory activity ETOA/POS (74.97%) with vitamin C standard,75.95% followed by 0.0625mg/ml, ETOA/POS (68.87%) inhibitory activity at 75.95% vitamin C standard. However, some of the extract showed a high percentage in antioxidant activity as expressed using vitamin C as standard.

Table 3: THE ANTIOXIDANT ACTIVITY, % INHIBITION AT DIFFERENT CONCENTRATION (mg/ml) COMPARE WITH VITAMIN C

Conc.mg/ml	PPT/DCM/POS	FILT/DCM/POS	ETOA/POS	MTH/POS	VIT.C
0.50	33.92	50.73	34.70	50.53	75.93
0.25	41.96	41.96	52.19	57.67	75.13
0.125	28.80	24.71	74.97	59.79	75.93
0.0625	18.57	18.13	68.87	56.35	75.93
0.0312	11-40	13.01	58.28	58.47	76.16

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Mineral Analysis

Table-4 revealed that the highest quantity of Calcium Ca: 6.1824mg/g was found in the leaf of *Pterocarpus Osun* while the least was Nickel Ni: 0.0013mg/g. The presence of mineral such as Calcium Ca, in the leaves can ensure adequate removal of anti-nutritional factors. High contribution of Potassium K and Magnesium Mg was found in the leaf.

The mineral composition in the leaf investigated showed the contents as in the order of Ca:6.1824mg/g>K:1.8407mg/g.>Mg:0.6656mg/g>Cd:0.2987mg/g>Fe:0.2974mg/g>Na:0.2505mg/g>Mn:0.1475mg/g>Cu:0.0587mg/g>Cr:0.0481mg/g>Zn:0.0218mg/g>Pb:0.0161mg/g>Ni:0.0013mg/g.

Table 4: MINERAL ANALYSIS

No.	Parameters	Conc.mg/g
1	Iron (Fe)	0.2974
2	Copper (Cu)	0.0587
3	Potassium (K)	1.8407
4	Magnesium (Mg)	0.6656
5	Chronium (Cr)	0.0481
6	Lead (Pb)	0.0161
7	Sodium (Na)	0.2505
8	Manganese (Mn)	0.1475
9	Cadnium (Cd)	0.2987
10	Zinc(Zn)	0.0218
11	Nickel (Ni)	0.0013
12	Calcium (Ca)	6.1824

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Potassium K act as a cofactor and has an active role in muscle excitability while Sodium Na element regulate the Osmotic and acid base balance of the body fluid system.

Antimicrobial screening

The antimicrobial activity of PPT/DCM/POS, FILT/DCM/POS, ETOA/POS and MTH/POS extract as in Table-5, showed sensitivity in *Staphylococcus aureus*. The extracts were sensitive in *E. coli* but resistant in MTH/POS. *Klebsiella pneumonia* showed sensitivity in PPT/DCM/POS, ETOA/POS and MTH/POS but resistant to FILT/DCM/POS.

The zone of inhibition of the extract against the test microorganism as shown in Table-6 occurred in the four extract at 20mm, 25mm, 23mm, and 26mm for *staphylococcus aureus* and extract of PPT/DCM/POS(25mm),FILT/DCM/POS(22mm) and ETOA/POS(23mm) were sensitive in *E. coli* but resistant in MTH/POS extract. The antimicrobial activities exhibited by *Pterocarpus osun* in this study may be attributed to the presence of alkaloids, flavonoids, and other bioactive compounds present in it, individually or in combination exhibit antibacterial activities. One of the mechanisms of action of alkaloids is intercalate into cell wall and DNA of bacteria. Alkaloids have been implicated for its detoxifying and antihypertensive properties in research carried out and flavonoids are found to be effective antimicrobial substances against a wide range of microorganism [21]

Table 5: ANTIMICROBIAL ACTIVITYOF PPT/DCM/POS, FILT/DCM/POS, ETOA/POS and MTH/POS

Test Organism.	PPT/DCM/POS	FILT/DCM/POS	ETOA/POS	MTH/POS
Methicillin resist staph aureus	R	S	R	S
Vancomycin resist	R	R	S	R
Staphylococcus aureus	S	S	S	S
Escherichia coli	S	S	S	R
Acinetobacter sp.	R	S	R	S

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Klebsiella pneumoniae	S	R	S	S
Salmonella sp	S	R	R	S
Heamophilus influenza	S	R	S	R
Streptococcus pneumonia	R	S	R	S

Key; S=Sensitive; R= Resistance

Table 6: ZONE OF INHIBITION OF THE EXTRACT AGAINST THE TEST MICROORGANISM.

Test Organism	PPT/DCM/POS	FILT/DCM/POS	ETOA/POS	MTH/POS
Methicillin resist staph aureus	0	23	0	25
Vancomycin resist enterococci	0	0	25	0
Staphylococcus aureus	20	25	23	26
Escherichia soli	25	22	23	0
Acinetobacter Sp.	0	27	0	28
Klebsiella pneumoniae	23	0	22	24
Salmonella sp	24	0	0	26
Heamophilus influenza	21	0	26	0
Streptococcus pneumonia	0	27	0	25

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Table 7: CONTROL AGAINST THE ORGANISMS

Organism	Sparfloxacin	Ciprofloxacin	Streptomycin
Methicillin resist staph aureus	S	R	R
Vancomycin resist Enterococci	R	S	R
Staphylococcus aureus	S	R	S
Escherichia coli	R	S	R
Acinetobacter sp	S	S	S
Klebsiella pneumoniae	S	R	S
Salmonella sp	R	S	R
Heamophilus influenza	S	R	R
Streptococcus pneumonia	S	R	R

Key; S= Sensitive; R= Resistance

Table 8: ZONE OF INHIBITION OF THE DRUG AGAINST THE TEST MICROORGANISM

Test Organism	Sparfloxacin	Ciprofloxacin	Streptomycin
Methicillin resist staph Aureus	30	0	0
Vancomycin resist Enterococci	0	29	0

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Staphylococcus aureus	32	0	30
Escherichia coli	0	37	0
Acinetobacter sp	27	31	29
Klebsiella pneumoniae	30	0	31
Salmonella sp	0	39	0
Heamophilus influenza	28	0	0
Streptococcus pneumonia	25	0	0

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