

Nutrients, Phytochemical, Antioxidant and Antimicrobial Analysis of *Pterocarpus osun* stem bark and leaf for their nutritional, medicinal capacity

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Abstract

Plants play a crucial role in human well-being and health. They provide some of the essential nutrients that humans require as well as act as medications to alleviate and cure various health problems. The purpose of this study is to look into the nutritional value, mineral composition, and the overall contributions of *Pterocarpus osun* to human nutrition and health. The result of the proximate analysis showed that protein is 9.52% in stem bark and 13.63% in the leaf, while the crude fiber in the stem bark is 37.89% and 46.03%, in the leaf. Ash, 6.74% and 7.46% in the stem bark and leaf respectively while carbohydrate content is 15.37% (stem bark) and 3.26% (leaf). Alkaloids, flavonoids, terpenoids, steroids, and tannins were detected in both organs of the plant tested. The mineral elements present include Ca, Mn, Fe, Ni, Mg, Zn, Cr, Co, Cd, Sulphur, and phosphorus. The antioxidant effect compared favorably well with that of the ascorbic acid used as standard. The extracts were screened for antimicrobial activities using eleven human pathogens. Each of the extracts successfully killed six microbes..

Keywords: Antioxidant, nutrients, secondary metabolites, pterocarpus osun, microbes

INTRODUCTION

Plants materials have been used for medicinal purposes throughout history. Their health benefits are associated with some of their chemical constituents, such as vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, and minerals (Calucci et al., (2003); Suhaj, (2006). They are sources of flavorings, aromatic compounds, and medicines. Plants have a profound impact on human well-being and health (Saidur Rahman et al., 2018). They provide some of the essential nutrients that humans require, such as carbohydrates, protein, fat, fiber, and minerals, as well as acting as medications to treat and alleviate their health problems (Begum, 2018). These nutrients play an important part in meeting the needs of the human body. As food, drugs, and nutritional supplements, humans employ a wide range of plants and plants' products (Sen & Samanta, (2014); Begum, 2018).

Plants are known to include essential nutrients such as mineral elements, lipids, proteins, fiber, carbohydrates, and plant chemical compounds such as phytochemicals or secondary metabolites, some of which can be used as therapeutic agents for human disease. Plants have traditionally been employed raw,

boiled, or in liquid combinations for the treatment of ailments without regard for their nutritional makeup, which is essential for the body's correct physiological functioning (Adnan et al., (2010); (Hussain, (2013). According to the World Health Organization, 80% of the world's population relies on plants and plant products to survive. Medicinal plants have been used in healthcare since time immemorial (Sofowora, Ogunbodede, & Onayade, 2013). Studies have been carried out globally to verify the efficacy of plants materials as therapeutic agents and some of the findings have led to the production of plant-based medicines. Because medicinal plants contain bioactive chemicals, they can be used as anticancer, antibacterial, anti-inflammatory, antioxidant, anti-malaria, and other agents. They do, however, provide nutrients for human health also.

The proximate, nutritional, and anti-nutritional assessments can be used to determine the therapeutic benefits and nutritional values of plants (Akpabio & Ikpe, 2013). The mineral makeup of the plant is determined by the ash content, which may have an impact on the plant's medicinal function (Tomescu et al., 2015.). Protein concentration in plant parts is important in the description of plant parts as nutrient value, classification, plant development, and plant

conservation (Hussain et al., 2011). A variety of medicinal plants are employed in the diet as a result of their therapeutic restorative benefits. As a result, evaluating medicinal plants for their nutritional contents can be quite useful in determining the usefulness of these specific medicinal plants in the treatment of health disorders.

Pterocarpus osun is regarded to have significant therapeutic powers in traditional medicine. The stem bark of *Pterocarpus osun* has been used in traditional medicine to treat diarrhea, dysentery, and gastrointestinal problems (Burkill, 1995). The bark of the *Pterocarpus* plant is used for tooth and mouth problems, while the bark resin is used as an astringent for severe diarrhea and dysentery (Gill, 1992). It's also been demonstrated to work in the treatment of fevers (Hutchinson *et al.* 1958). The dry leaf is used in traditional black soap made from ash from burned cocoa pods and palm oil, while the heartwood, bark, and roots are ground into a paste and used as a skin cosmetic. It has been used to treat rheumatism, eczema, gonorrhea, candidiasis, and acne (Uphoff, 1959). Traditional treatments for sickle-cell disease and amenorrhea include *P. osun* stem (Uphoff, 1959).

Other species, such as *Pterocarpus marsupium*, are high in tannins and flavonoids, and are thus used as an astringent, anodyne, cooling, and regenerating agent, as well as for the treatment of leprosy, leucoderma, toothache, fractures, diarrhea, passive hemorrhage, and dysentery, as well as bruises and diabetes. Rheumatoid arthritis, gout, diabetic anemia, indigestion, asthma, cough, hair discoloration, bronchitis, ophthalmic problems, elephantiasis, and erysipelas are among the conditions for which it is prescribed (Rahman et al., 2018). Leucoderma, elephantiasis, diarrhea, cough, hair discoloration, and proctalgia have all been treated with *P. marsupium* in the past (Mankani et al., 2005). It is nontoxic and useful in jaundice, fever, wounds, diabetes, stomachache, and ulcer (Jung et al., 2006). The heartwood, bark, and roots are ground into a paste and applied to the skin as a cosmetic (www.prota.org) (www.prota.org).

The antioxidant properties, as well as the depigmenting effect, have been established (Krumbeigel, 1948). According to investigations, the wood contained red pigments called santarubin and santalin, which can be utilized as histology stains (Osugwu, 2008). The powdered stem protects the freshly severed umbilical cord against infection. Rheumatism, eczema, gonorrhea, candidiasis, and acne have all been treated with it (Gill, 1992). *Pterocarpus spp.* is an example of a plant that has

been used to treat type 2 diabetes (Mukherjee, Maiti, Mukherjee, & Houghton, 2006). *Pterocarpus spp.* stem bark powder has also been used to cure diarrhea, and the wood powder has been administered externally to treat inflammations, headaches, mental aberrations, and ulcers (Krishna Veni & Srinivasa Rao, 2000).

The greatest activity against *Enterobacter aerogenes*, *Alcaligenes faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus* was found in the stem bark extract (Manjunatha, 2006). The heartwood, bark, and roots are mashed into a paste and used as skin cosmetics, while the dry leaf is an ingredient in traditional black soap made from the ash of burned cocoa pods and palm oil. This study is therefore aimed at analyzing the leaf and stem bark's nutritional potential as well as their disease-relieving qualities.

METHODOLOGY

Proximate analysis

For proximate analysis, the AOAC (1990) method was employed, which included measurements of moisture content, ash content, crude fat, crude protein, carbohydrate, and energy. The gross energy content was calculated using the Atwater's conversion factors: 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat, and 16.7 kJ/g (4 kcal/g) for carbohydrates, and expressed in calories (Guyot *et al.*, 2007). Carbohydrate was determined by using the formula:

$$\text{Carbohydrate (\%)} = 100 - (\% \text{ crude lipid} + \% \text{ crude fiber} + \% \text{ Ash} + \% \text{ Protein} + \% \text{ Moisture})$$

Mineral analysis

Two grams (2 g) of each sample were weighed into a beaker, followed by 20mL of nitric acid. This was heated for 30 minutes on the hotplate at 60 degrees Celsius. After that, it was taken off the heat and left to cool. In a volumetric flask, the solution was diluted with distilled deionized water and made up to 50mL. In the same way, a blank was made and poured into a polypropylene bottle. Using acceptable working standards, the samples were analyzed for metals on a Thermo Scientific iCE 3000AA02134104 Atomic Absorption Spectrometer (Fadeyi et al., 2020) using the lamps for Ca, Mn, Fe, Ni, Mg, Zn, Cr, Co, and Cd. The colorimetric method of vanadomolybdate was used to determine phosphorus and sulphur (Ologhobo & Fetuga, 1983).

Qualitative phytochemical screening

Test for Phenols: This test was performed using the method of Sofowora (1993). 2mL extract was taken in a beaker to which, 2mL of ferric chloride solution was added. A deep bluish-green solution indicated the presence of phenols.

Test for Terpenoids: Salkowski test was performed by using the method of Edeoga *et al.* (2005). 5mL of aqueous extract was mixed in 2mL of chloroform. Then 3mL of concentrated sulfuric acid was added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids.

Test for Saponins: This was performed by using the method of Edeoga *et al.* (2005). 2g of the powdered sample boiled in 20mL of distilled water in a water bath and filtered the solution. Then 10mL of the filtrate was mixed with 5mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shakes vigorously which leads to the formation of the emulsion; indicating the presence of saponins.

Test for Flavonoids: This test was performed using the method of Harborne (2005). 1g powdered sample was heated with 10mL ethyl acetate over a steam bath (40–50°C) for 5min. The filtrate was treated with 1mL dilute ammonia. A yellow coloration demonstrated a positive test for flavonoids.

Test for Alkaloids: This was performed by using the method of Harborne (2005). 1g powdered sample was extracted with 5mL methanol and 5mL of 2N hydrochloric acid. The filtrate was treated with Meyer's and Wagner's reagents. The samples were scored positive on the basis of turbidity.

Test for Glycosides: Kellar–Kiliani test was performed by using the method of Parekh and Chanda (2007). To 2mL of the filtrate, 1mL of glacial acetic acid was added. Then 1mL of ferric chloride was added with 1ml concentrated sulfuric acid. Green-blue coloration of the solution indicates that glycoside is present.

Test for Tannins: The test was performed by using the method of Kumar *et al.* (2007). Alcoholic ferric chloride solution (10%) was added in 2-3mL of methanolic extract (1:1). The development of the dark blue color of the solution indicated the presence of tannins.

Test for Steroids: Identification of steroids was done by adopting the method described by Edeoga *et al.*, (2005). To 1mL of extract, 2mL acetic anhydride, and 2 mL concentrated sulfuric acid was added, colour change from blue to dark green indicates the presence of steroids.

Determination of Anti-nutrient/Quantitative Phytochemicals

The existence and quantity of phenols (AOAC, 1995), alkaloids (Manjunath *et al.*, 2012), tannins (Van-Burden and Robinson, 1981), flavonoids (Vabkova and Neugebauerova, 2012), and saponins (Obadoni and Ochuko, 2001) were investigated.

Free radical scavenging activities

The antioxidant activity was measured using a slightly modified method developed by Brand-Williams *et al.* (1995), for discoloration of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) in methanol. The extract was evaluated at the following concentrations: 1000, 500, 250, 125 and 62.5µg/mL, and the absorbance was measured at 517nm after incubation for 30 minutes in the dark. Ascorbic acid was used as standard at the same concentrations prepared for the extracts. Blank solutions were prepared with the same amount of methanol. The following Equation 1 was used to compute the radical scavenging activities:

$$\text{Percent Inhibition} = \frac{B_{\text{abs}} - S_{\text{abs}} \times 100}{B_{\text{abs}}} \quad (1)$$

Where B_{abs} = absorbance of blank solution;
 S_{abs} = absorbance of the sample

The antioxidant capacity was then evaluated by determining the IC_{50} from non-linear regression.

Data Analysis

The descriptive statistics described by Olawuyi (1996) was used to analyze all of the data collected. The mean and standard deviation were calculated as statistical values.

Antimicrobial Screening

Human pathogenic microbes used were obtained from the Medical Microbiology Department, Ahmadu Bello University, Zaria. The extracts were screened using the diffusion method with Mueller Hinton agar as the bacteria' growth medium. The medium had been prepared by sterilizing it at 121degrees Celsius. 15 minutes later, it was put into sterile Petri plates and allowed to cool and solidify. The sterilized medium was planted with 0.1mL of the test microorganisms' standard inoculums. A sterile swab was used to disseminate each inoculum equally across the surface of the media, and a well was cut in the center of each inoculated medium with a standard cork-borer of 6mm diameter. The extract was dissolved in 0.1mL of water at concentrations of 20 mg/mL, and then

injected into the well on the inoculation medium. After a 24-hour incubation period at 37 °C, the plates of the media were examined for the inhibited zones. Using the broth dilution procedure, the extract's minimum inhibitory concentration (MIC) was measured. Mueller Hinton broth was made, and 10ml was poured into test tubes.

The broths were sterilized at 121 °C for 15 minutes and then allowed to cool. The concentration was calculated using McFarland's turbidity standard scale number 0.5. The test microorganism was injected and cultured at 37 °C for 6 hours after the normal saline was produced and 10ml poured into a sterile test tube. The test microbe was diluted in normal saline until it became as turbid as Mc-scale Farland's by visual comparison; at this point, the test microbe has a consensus. The crude methanol extracts of leaf and stem bark were serially diluted two times in sterile broth to generate concentrations of 20mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL respectively.

RESULTS AND DISCUSSION

Proximate analysis

The proximate composition of plants provides valuable information about their medicinal and nutritional qualities. The nutritional analysis result as presented in Table 1 and Figure 1, indicated that both stem bark and leaf contain high fibre, lipids, proteins, and ash in that order. Carbohydrates in the stem bark was significantly higher than that of the leaf. The protein, ash, and lipid contents support the nutritional importance of the plants. Plants have been reported to play significant roles in drug discovery

Phytochemical screening

The result of the analysis of the qualitative, quantitative phytochemicals and antinutritional analysis of *P. osun* secondary metabolites (Tables 2 and 3) showed that both organs of *P. osun* under study contain alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, cardiac glycoside, phenols present. This shows high level of their possible medicinal and dietary values. The saponin content of plant components, notably stem bark (1.216±0.09 mg/g), suggests that the plant can be utilized to decrease blood cholesterol by inhibiting its reabsorption, making it useful for cardiovascular disease prevention (Osagie and Eka, 1998).

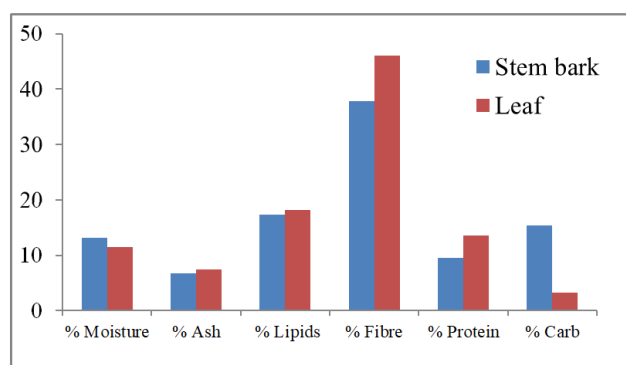


Figure 1. Bar chart showing the comparison of the nutritional values of the stem bark and the leaf of *P. osun*

The saponin content of the samples may also increase their usefulness, especially the stem bark, in lowering the risk of human cancer since it has been documented that saponin has antitumor and antimutagenic properties (Rao & Sung, 1995).

Table 2. Qualitative Phytochemical analysis

Table 1. Proximate analysis of *P. osun*

	% Moisture	% Ash	% Lipid	% Fibre	% Protein	% Carb.	Energy (Kcal)
SMB:	13.18±0.16	6.74±0.24	7.31±0.27	37.89±0.27	9.52±0.14	15.37±0.90	255.26
Leaf:	11.46±0.19	7.46±0.16	8.17±0.27	46.03±0.81	13.63±0.37	3.26±0.62	231.09

Note: SMB = Stem bark, Carb = Carbohydrate was obtained by difference
The given values are mean±SD of three different determinations.

and have continue to receive diligent attentions because of their inherent bioactive component attributes such as antioxidants, anticancer, anti-inflammatory, antibacterial. Secondary metabolites are the chemical substances responsible for these biological activities of plants.

Metabolites	Stem bark	Leaf
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Phenols	+	+
Cardiac glycoside	+	+
Saponins	+	+
Steroids	+	+
Terpenoids	+	+

Note: + = Present, - = Absent

Table 3. Anti-nutrient/Quantitative phytochemical analysis of *P. osun*

Metabolites	Leaf (mg/g)	Stem Bark (mg/g)
Tannins	1.318±0.21	1.521±0.17
Alkaloids	0.690±0.14	0.824±0.25
Flavonoid	1.342±0.32	0.174±0.61
Saponins	0.830±0.18	1.216±0.09
Phenols	0.208±0.22	0.479±0.30
Phytate	0.342±0.17	0.256±0.20
Oxalate	0.928±0.24	0.680±0.16

The given values are mean±SD of three different determinations

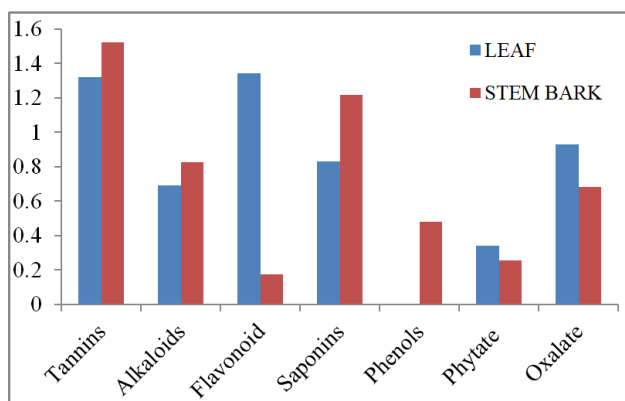


Figure 2. Quantitative values of the stem bark and leaf of *P. Osun*

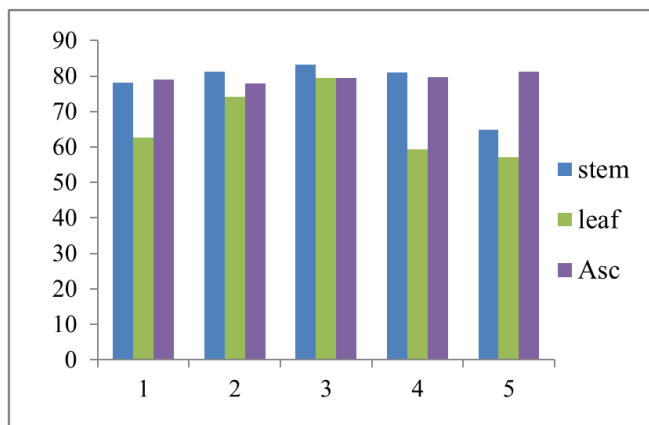


Figure 3. % Antioxidant activities of the stem bark and the leaf of *P.osun* with that of ascorbic acid (Stem: stem bark, Asc:ascorbic acid)

The result of the quantitative phytochemical analysis of the plant parts (Table 3 and Figure 2) showed that the stem bark and the leaf have tannins values as 10.220 mg/g and 10.100 mg/g respectively.

Table 4. Antioxidant activities measurement

Concentration (µg/mL)	%AA Stem Bark	%AA Leaf	%AA Ascorbic
62.5	64.80±0.17	57.21±0.20	81.27±0.31
125	81.10±0.08	59.30±0.24	79.77±0.22
250	83.12±0.15	79.36±0.15	79.43±0.16
500	81.30±0.11	74.16±0.21	77.69±0.16
1000	78.21±0.13	62.60±0.32	79.05±0.26

Note: AA – Antioxidant Activity

Studies have shown that tannins possess antidiarrhoeal (Rani et al., (1999); Mbagwu & Adeyemi, (2008); Amabeoku, (2009); Tian et al., (2009); Yang et al., (2017); Bonelli et al., (2018); anti-inflammatory (Wijesinghe et al., 2013); antiparasitic (Minho, Gennari, Amarante, & Abdala, 2010); and antimicrobial activities (Dall’Agnol et al., 2003); Corrales, Han, & Tauscher, (2009); Tong, He, Fan, & Guo, (2022).

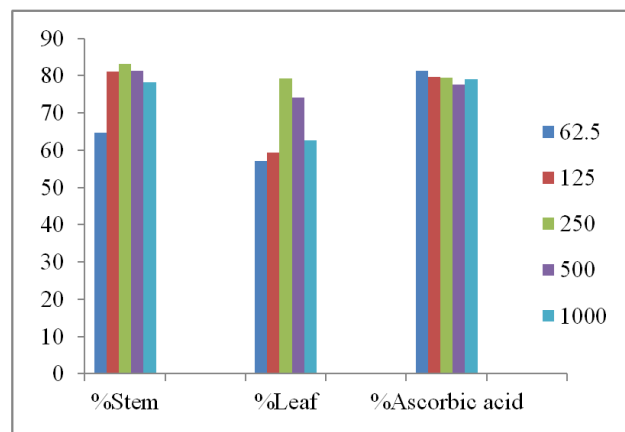


Figure 4. % Antioxidant activities of stem bark, leaf and ascorbic acid at different concentrations

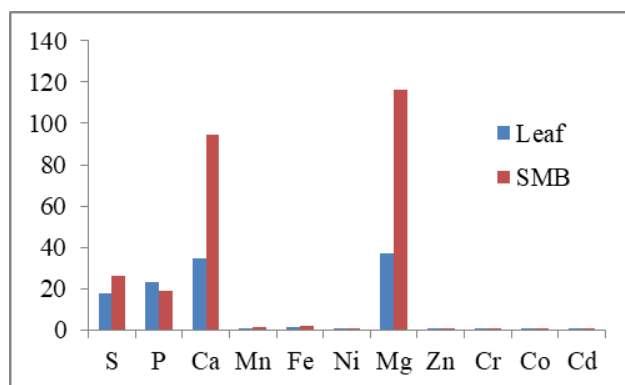


Figure 5. Elemental composition of the stem bark (SMB) and the leaf of *P.osun*

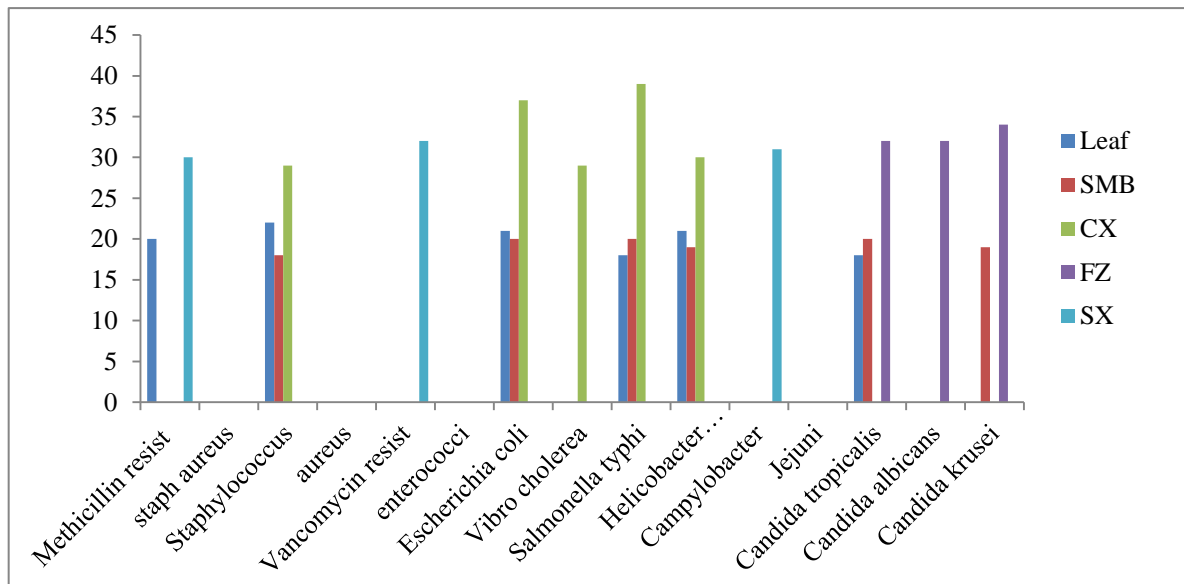


Figure 6. Zones of inhibition of extracts and control drugs

Table 5. Mineral analysis of *P. osun* stem bark and leaf (mg/Kg)

Elements	S	P	Ca	Mn	Fe	Ni	Mg	Zn	Cr	Co	Cd
Leaf	17.77	23.33	34.47	0.26	1.10	0.27	36.84	0.36	0.24	0.22	0.12
Stem bark	25.85	19.00	94.40	1.54	1.67	0.27	116.45	0.20	0.58	0.22	0.12

Table 6. Zones of Inhibition of extracts and control drugs, Qualitative antimicrobial, Minimum inhibitory concentration, and Minimum Bacteria/Fungal concentration

Test organisms	Zones of Inhibition (mm)					Qualitative		MIC (mg/mL)		MB/FC (mg/mL)	
	Leaf	SMB	CX	FZ	SX	Leaf	SMB	Leaf	SMB	Leaf	SMB
<i>Methicillin resist</i>	20	0	0	0	30	S	R	5.0	-	20	-
<i>staph aureus</i>	22	18	29	0	0	S	S	5.0	10.0	20	20
<i>Staphylococcus aureus</i>	0	0	0	0	32	R	R	-	-	-	-
<i>Vancomycin resist</i>	0	0	0	0	32	R	R	-	-	-	-
<i>enterococci</i>	21	20	37	0	0	S	S	5.0	5.0	20	20
<i>Escherichia coli</i>	0	0	29	0	0	R	R	-	-	-	-
<i>Vibro cholerea</i>	18	20	39	0	0	S	S	10.0	5.0	20	20
<i>Salmonella typhi</i>	21	19	30	0	0	S	S	5.0	10.0	20	20
<i>Helicobacter pylori</i>	0	0	0	0	31	R	R	-	-	-	-
<i>Campylobacter</i>	0	0	0	0	31	R	R	-	-	-	-
<i>Jejunii</i>	18	20	0	32	0	S	S	10.0	5.0	20	20
<i>Candida tropicalis</i>	0	0	0	32	0	R	R	-	-	-	-
<i>Candida albicans</i>	0	0	0	32	0	R	R	-	-	-	-
<i>Candida krusei</i>	0	19	0	34	0	R	S	-	10	-	20

The result of the quantitative phytochemical analysis of the plant parts (Table 3 and Figure 2) showed that the stem bark and the leaf have tannins values as 10.220 mg/g and 10.100 mg/g respectively. Studies have shown that tannins possess antidiarrhoeal (Rani et al., (1999); Mbagwu & Adeyemi, (2008); Amabeoku, (2009); Tian et al., (2009); Yang et al., (2017); Bonelli et al., (2018); anti-inflammatory (Wijesinghe et al., 2013); antiparasitic (Minho, Gennari, Amarante, & Abdala,

2010); and antimicrobial activities (Dall’Agnol et al., 2003); Corrales, Han, & Tauscher, (2009); Tong, He, Fan, & Guo, (2022). The appreciable quantities of tannin in samples may confer the aforementioned properties on the samples. Also, flavonoids and phenols quantities were significant. Phenolic compounds are important antioxidant compounds.

Mineral analysis

The result of mineral analysis in Table 5 and Figure 5 indicates the presence of Sulphur (S), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Manganese (Mn), Iron (Fe), Nickel (Ni), Zinc (Zn),

functioning by delivering therapeutic effects and energy-rich nutrients for the growth and development of the human body. In light of the aforesaid findings, *P. osun* stem bark and leaf may have the capacity to supply nutrients and medicines which are essential for

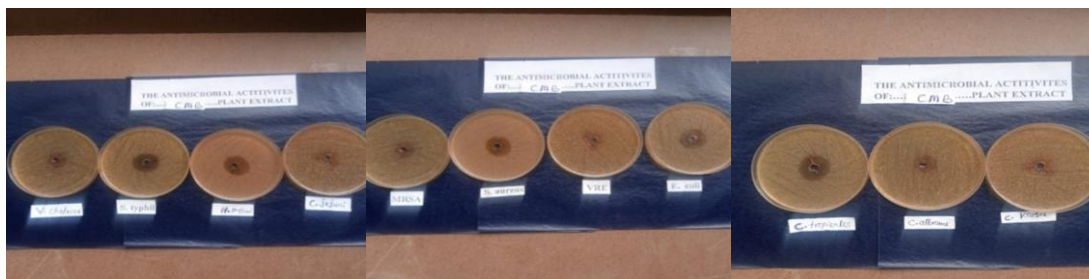


Figure 7. Zones of inhibition for the stem bark

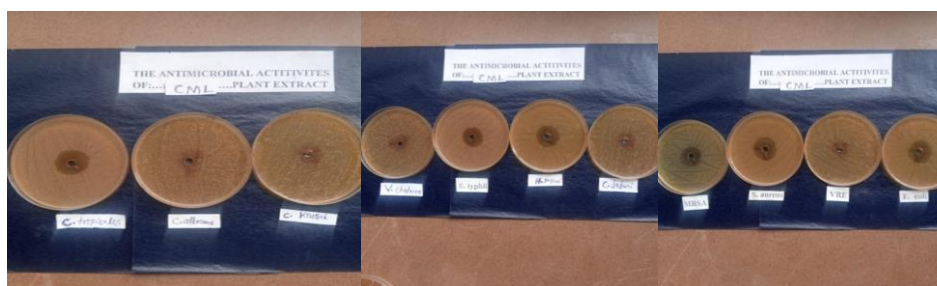


Figure 8. Zones of inhibition for the leaf extract

Chromium (Cr), Cobalt (Co), and Cadmium (Cd) at different concentrations. A higher concentration of Ca and Mg (94.4 & 116.45mk/kg) was observed in *P. osun* stem bark. This is in agreement with the findings of Orishadipe *et al.* (2015) on the mineral content of leaf extract of *P. osun*.

Antimicrobial analysis

In Table 6 and Figures 6, 7 and 8, the results of the antimicrobial activities of both organs of *P. osun* are displayed. Eleven microbes were tested for activities and six of them were sensitive while five resist. This plant's parts are effective against multidrug-resistant microbes like *S. aureus*, *E. coli*, *VRE*, *C. tropicalis*, *C. krusei*. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolites of the plant such as tannin and phenol (Janssen, Scheffer, & Svendsen, (1987); Saxena, McCutcheon, Farmer, Towers, & Hancock, (1994); Corrales et al., (2009); Tong et al., (2022).

CONCLUSION

The current study concludes that *Pterocarpus osun*, stem bark, and leaf can play essential roles in the preservation of a healthy life and regular body

human wellness and maintenance of health, growth, and development of the body.

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