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Phytochemical, Antioxidant and Antimicrobial Analyses of *Pterocarpus mildbraedii* Stem Extracts

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ABSTRACT

Phytochemical screening, antioxidant and antimicrobial activities of *n*-hexane, ethyl acetate and methanol extracts of *Pterocarpus mildbraedii* stem were studied in this research. *Pterocarpus mildbraedii* is used in folk medicine to treat fever, convulsions and respiratory disorders. The air-dried and milled plant materials were extracted with soxhlet apparatus at a temperature of 80 ^{0}C using *n*-hexane, ethyl acetate and methanol successively. The preliminary screening of the extracts was carried out using standard methods, and the free radical scavenging capacity using 2, 2 diphenyl-1-picryhydrazyl (DPPH) was determined to evaluate the antioxidant activity. Likewise, the antimicrobial screening was also carried out using the Broth dilution method. Phytochemical screening of the extracts (*n*-hexane, ethyl acetate and methanol) revealed the presence of alkaloids, tannins, flavonoids, steroids, terpenes and phenol. However, tannin was not found in the *n*-hexane extract, while saponin and cardiac glycoside were also absent in all the extracts. The extracts were concentration dependent on the DPPH free radicals scavenging. The IC_{50} values were 0.8546, 0.8953 and 0.9081 µg/mL for the methanol, *n*-hexane and ethyl acetate extracts respectively. The antimicrobial screening showed that the methanol extract was still active at a concentration of 12.5 mg/mL. The result of this study suggests that the stem of *Pterocarpus mildbraedii* may be a good source of antioxidants and antimicrobial agents. Hence, the research justifies the use of *Pterocarpus mildbraedii* in traditional medicines.

1. INTRODUCTION

Nigeria is endowed with a variety of medicinal plants whose seeds, bark, roots, and leaves are used to treat a variety of illnesses. In Eastern Nigeria, the leaves of *Pterocarpus mildbraedii* Harms are referred to as "Oha" and are one of the popular vegetables there. The fresh, edible parts of herbaceous plants known as vegetables can be either raw or cooked (Fayemi, 1999, Dhellot *et al*., 2006, Hassan *et al*., 2007). Vegetables are important for preserving the body's alkaline reserve. They are mostly regarded

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for their rich mineral, vitamin, and carbohydrate contents (Awol, 2014). Carotene, ascorbic acid, riboflavin, folic acid and minerals including calcium, iron and phosphorus are all abundant in vegetables (Fasuyi, 2006). Additionally, they have phytochemicals or minerals that decrease their bioavailability (Akindahunsi and Salawu, 2005). Some anti-nutritional chemicals, according to Aletor and Adeogun (1995), have protective effects, which allows them to serve a dual goal of decreasing some important nutrients and safeguarding the body from a variety of biochemical, physiological, and metabolic diseases. Vegetables can include edible fruits, seeds, stems, leaves, roots, and stems. Each group has a unique contribution to diet (Chinma and Igyor, 2007). However, several affordable green vegetables still have untapped nutritional and antinutritional potential that needs more research. The leaves of *Pterocarpus mildbraedii* Harms are one of these greens. The emergence of new ailments in diverse forms and an increase in drug resistance to synthetic substances have led to the creation of natural goods for medical purposes. Due to the chemical compounds, they contain, medicinal plants have a significant impact on people's health. These compounds have a specific pharmacological effect on the human body (Liu and Wang, 2008). Prior to now, the focus on using medicinal herbs had been on disease treatment rather than prevention. However, there are numerous recent reports of research on the use of medicinal plants and their ingredients in illness prevention in the literature. This paper now reports the results of the analysis of the phytochemicals, antioxidant, and antimicrobial activities of the *Pterocarpus mildbraedii* stem extracts.

2. MATERIAL AND METHODS

2.1 Collection of plant and authentication

Pterocarpus mildraedii plant was collected from the Botanical Garden, University of Ibadan in Oyo state, Nigeria. The plant was authenticated and identified at the Herbarium unit of the Botany department at the University of Ibadan, Oyo state.

2.2 Processing of plant material

The stem of the plant was air dried for about 14 days inside the laboratory to remove moisture and pulverized and later milled to mesh sizes.

2.3 Preparation of the plant extract

320 g of the dried and milled plant (*Pterocarpus mildbraedii* stem) material was extracted with a hot (Soxhlet) extractor successively at the temperature of 69 ^{o}C. Hexane, ethyl acetate and Methanol which are the solvent were allowed to remain in contact with the plant material for about 12 hrs, the extract was evaporated to dryness by using a rotary evaporator and 5.3 g, 7.2 g and 10 g of the extracts were obtained respectively.

2.4 Phytochemical

The extracts were analyzed for the presence of alkaloids, saponins, tannins, steroids, cardiac-active glycoside, reducing sugar, flavonoids, resins and phenols.

Test for Alkaloids

0.2 g of extract was shaken with 1% of HCl for two minutes. The mixture was filtered and drops of Dragendroff's reagent were added. The formation of a precipitate indicated the presence of alkaloids.

Test for Saponins
0.2 g of extracts was shaken with 5 mL of distilled water in a test tube. Frothing which persists on warming was taken as evidence of saponins.

Test for Tannins
0.2 g of extract was stirred with distilled water and filtered. Ferric chloride was added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence of the presence of tannins.

Test for Steroids (Salkowski's Test)
0.2 g of the extracts were dissolved in 2 mL of chloroform. Concentrated sulfuric acid was gradually added to form a layer. A reddish brown colour at the interface indicated the deoxy sugar characteristics of cardenolides.

Test for Cardiac-Active Glycosides (Keller Kilian's Test)
0.2 g 0f extract was dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride solution followed by the addition of 1 mL of concentrated sulfuric acid. A brown ring at the interface confirmed the presence of cardiac glycosides.

Test for Reducing Sugars
0.2 g of the extracts were shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for two minutes. An orange precipitate on boiling with Fehling's solution indicated the presence of reducing sugars.

Test for Flavonoids
A little amount of magnesium powder and a few drops of concentrated hydrochloric acid were added to 3 mL of the extracts. A red or intense red colouration indicated the presence of flavonones.

Test for Resins
5 mL of copper acetate solution was added to 5 mL of the extracts. The resulting solution was shaken vigorously and allowed to separate. A green-coloured solution is evidence of the presence of resins.

Test for Phenols
0.2 g of the extracts were dissolved in a ferric chloride solution. A green or dirty green precipitate indicated the presence of a phenolic compound.

2.5 Scavenging effect on DPPH
According to the procedure of Oloyede *et al*. (2010) and Odeja *et al*. (2021) which was employed to determine the radical scavenging. A solution of 2, 2 diphenyl -1-picrylhydrazyl (0.5 mM) was prepared in methanol. The concentration of DPPH was determined by absorption of 517 nm using UV/V is spectrophotometer. The sample at 1 mL, 0.5 mL, 0.25 mL and 0.125 mL were added to 2, 2 diphenyl -1-picrylhydrazyl (DPPH) solution. The decrease in absorption of DPPH at about 517 nm was measured spectrophotometrically after 10 mins of incubating against a blank containing the sample in methanol without DPPH. All these steps were performed in triplicate. The percentage for 2, 2 diphenyl-1-picryhydrazyl scavenged by the extract was calculated by using the formula below:

$$\% \text{ Inhibition } (\%I) = \frac{Ac - As}{As} \times 100 \quad (1)$$

Where A_c is the absorbance of the control

and A_s is the absorbance in the presence of the sample of extract and standard. For the 50 % Inhibitory Concentration (IC_{50}) evaluation the extracts and reference standard were evaluated and graphs showing the concentration of the test samples (*n*-hexane, ethyl acetate and methanol extracts, ascorbic acid and butylated hydroxylanisole versus % Inhibition (DPPH reduction) were plotted and shown in Figs. 1a, b, c, d and e.

2.6 Antimicrobial screening (Broth dilution)

The microbes used were suspended in suitable nutrition media and were poured into a sterile petri-dish and allowed to incubate for 24 hrs at 37 °C. Suitably cut circular filter paper pieces containing 100, 50, 25, 12.5 and 6.25 mg/mL antibiotic solutions test samples and standards were introduced into the media. Then the pieces were placed on the nutritional microbial media all over with suitable gaps in between and incubated again. After 24 hrs of incubation, the plates were removed and the diameter of the zone of inhibition of test and standard samples was measured in millimeters. By comparing the areas of the zone of inhibition of test extracts with the standard, the concentration and potency of test samples were determined.

2.6.1 Preparation of graded concentration sample

1000 mL of each sample was weighed and dissolved into 5 mL of the solvent of the extraction in other to obtain proper dissolution. From the 200 mg/mL solution, 2.5 mL was taken into another sample bottle and 2.5 mL of solvents was added to give 100 mg/mL, from this, 2.5 mL is taken into another sample bottle and 2.5 mL of solvents was added to give 50 mg/mL. From the 50 mg/mL solution, 2.5 mL was taken into another sample and 2.5 mL of solvents was added to give 25 mg/mL solution. A similar procedure was followed to obtain the 12.5 mg/mL and 6.25 mg/mL concentrations using the dry filter paper dispersion method.

2.6.2 Organisms

Bacteria: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruguinosa, Salmonella typhi, Klebsiella pneumoniae.* Fungi: *Candida albicans, Aspergillus niger, Penicillum notatum, Rhizopus stolonifera,* Methanol for methanolic extract, ethyl acetate for ethyl acetate extract and hexane for hexane extract. **+ve:** Positive control; Gentamicin 10 µg/mL (bacterial) and Tioconazole 30 % (fungi).

2.6.3 Minimal inhibitory concentrations

All microbiological experiments were conducted under anaerobic conditions. Following the suggested techniques from the Clinical and Laboratory Standards Institute (2006), MIC studies were carried out in 96-well microplates. The extracts (200 mg) were mixed in 40 L of dimethyl sulfoxide to create a stock solution that had 40 mg of extract per mL before being raised to 5 mL with sterile RCM that contained 1% Tween 80. RCM was used to serially dilute an essential oil stock twice, resulting in final concentrations ranging from 20 to 0.625 g/mL. The microplate wells were filled with the diluted samples (100 L) and thoroughly mixed with a micropipette. Sterile RCM alone in conjunction with dimethyl sulfoxide served as the negative control (DMSO). In addition, gentamycin (10

g/mL for bacteria) and tioconazole (0.07 g/mL for fungi) were utilized as positive controls. Aseptic conditions could be determined because the control wells contained sterile RCM but no inoculum. The inoculated microplates underwent a 48-hour anaerobic incubation at 36 ^{o}C. By adding 10 L of a sterile 0.5 percent aqueous solution of triphenyltetrazolium chloride (TTC, Sigma-Aldrich) and incubation at 36 ^{o}C for 30 minutes, the bacterial growth was confirmed (Radeallia *et al*., 2016; Eloff) (1998). Using living bacteria, pink/red 1,3,5-triphenyl formazan was created from yellow TTC (TPF). The assays were carried out in triplicate.

2.6.4 Minimum bactericidal concentrations and Minimum fungicidal concentrations

MBCs and MFCs were assessed by inoculating the test mixtures from the wells showing no microbial growth onto the surface of sterile Shahidi-Ferguson Perfringens agar medium, in accordance with the Ministério da Agricultura, Pecuáriae Abastecimento's recommendations as described by Radaelli *et al*. (2016). After being incubated anaerobically for 24 hours at 36 ^{o}C in an oven, the plates were exposed to visual inspection. If there was no microbial growth on the medium, it was likely that the essential oil sample had bacteriostatic and fungistatic activities because it had bactericidal and fungicidal effects.

3. RESULTS AND DISCUSSION

3.1 Phytochemicals Screening of Pterocarpus mildbraedii stem extracts

Evaluation of their biological, dietary, and industrial applications pivots on the discovery of phytochemicals in the stem of *Pterocarpus mildbraedii*. The phytochemical screening of methanol, *n*-hexane, and ethyl acetate extracts of *Pterocarpus mildbraedii* stem extracts shows the presence of flavonoids, alkaloids, tannins, phenol and steroids. Tannin was absent in the hexane extract of *Pterocarpus mildbraedii* (Table 1). Earlier phytochemical screening of the leaf extract of *Pterocarpus mildbraedii* was investigated by Usunobun and Igwe (2016); leaf extract contains saponins, flavonoids, alkaloids, and tannins. The presence of flavonoids in *Pterocarpus mildbraedii* stem extracts suggests that the extract have the potential of exhibiting antioxidant property. In addition to their antioxidant properties, flavonoids protect against cancers, free radicals, platelet aggregation, bacteria, ulcers, viruses, and ulcerative colitis. Through their interference with the enzyme that makes estrogen, flavonoids lowered cancer. They lessen the development of edema and stop platelet stickiness, which then prevents platelet aggregation (Okwu and Ndu, 2006). *Pterocarpus mildbraedii* may be used to cure ulcers and to hasten the healing of wounds and cuts since it contains tannins. Alkaloids have been found in the stem of *Pterocarpus mildbraedii*, which may boost analgesic and anti-inflammatory potentials as well as disease resistance and stress tolerance. The bitter and sweet tastes of alkaloids are well known. They had been used as potent analgesics due to their similarity to neurotransmitters. Additionally, they are anti-leukemic and anti-malarial (Angela and Graham, 1991). Saponins, which are significant dietary and nutritional reserves (Chinedu *et al*., 2011), were found in the stem of *Pterocarpus mildbraedii*. The

nature of saponins is glycosidic (Sodipo *et al*., 2008). They can treat upper respiratory tract infections thanks to their expectorant activity. Cheek (1971) asserts that saponins play a function in the management of hypercholesterolemia. Alkaloids, saponins and tannins are significant components of many antibiotics used to treat typical pathogenic strains, according to Kubmarawa *et al*. (2007).

Table 1: Phytoconstituents of *Pterocarpus mildbraedii* stem extracts

Phytochemicals	**MPM**	**HPM**	**EPM**
Alkaloids	P	P	P
Tannin	P	A	P
Flavonoid	P	P	P
Saponin	A	A	A
Cardiac glycoside	A	A	A
Steroids	P	P	P
Phenol	P	P	P

Key: Hexane extract of Pterocarpus mildbraedii (HPM), Ethyl acetate of Pterocarpus mildbraedii (EPM), Methanol extract of Pterocarpus mildbraedii (MPM), P = Present, A = Absent.

3.2 In vitro Antioxidant activity of Pterocarpus mildbraedii stem extracts

The *in vitro* antioxidant activity was accessed using 2, 2-di(4-tert-octylphenyl)-1-picrylhydrazyl radical (DPPH˙). Fig. 1a - 1e revealed that all extracts displayed high antioxidant properties. Notwithstanding, the *P. mildbraedii* stem extracts contain several phytoconstituents with distinct antioxidant properties. Since the radical molecule is stable, the DPPH* test is a reliable, simple and affordable way to assess the antioxidants' capacity to scavenge free radicals (Brand-Williams *et al*., 1995; Sanchez-Moreno *et al*., 1998). The assay's process is based on how the antioxidant agent reduces the radical, either by donating an electron or by reducing DPPH to its reduced form, DPPH-H, a stable diamagnetic molecule, changing its colour from purple to yellow (Olaoluwa *et al*., 2018). It was observed that the stem extract *P. mildbraedii* exhibited significant antioxidant activity at the tested concentrations (Fig. 1) comparable to the reference standard ascorbic acid and BHA. The order of activity based on the IC_{50} is as follows: BHA (0.7112 mg/mL), ASC (0.8086 mg/mL), MPM (0.8546 mg/mL), HPM (0.8953 mg/mL) and EPM (0.9081 mg/mL). The phytochemicals found in the *P. mildbraedii* stem extract may be the cause of the considerable antioxidant activity seen in this investigation.

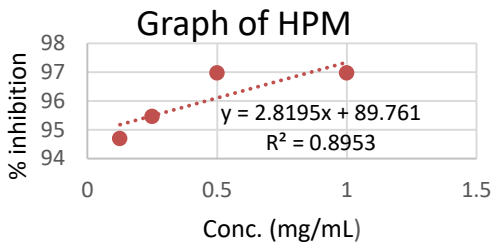

Fig. 1a: Plots of inhibition against concentration for Hexane extract of *Pterocarpus mildbraedii* (HPM)

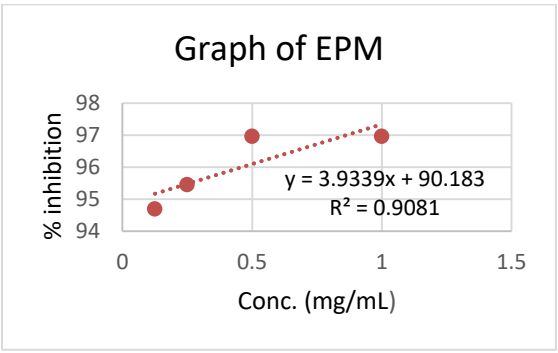

Fig. 1b: Plots of inhibition against concentration for Ethyl acetate extract

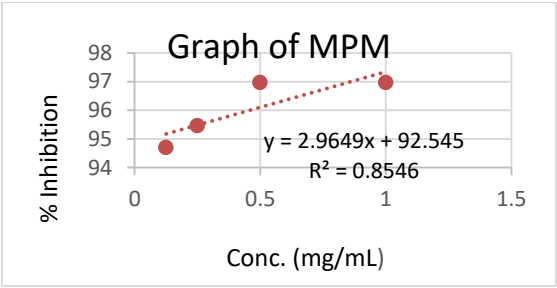

Fig. 1c: Plots of inhibition against concentration for Methanol extract of *Pterocarpus mildbraedii* (MPM)

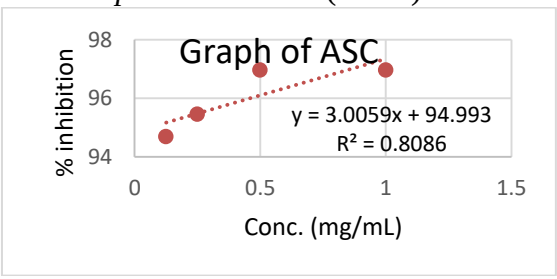

Fig. 1d: Plots of inhibition against concentration for Ascorbic acid (ASC)

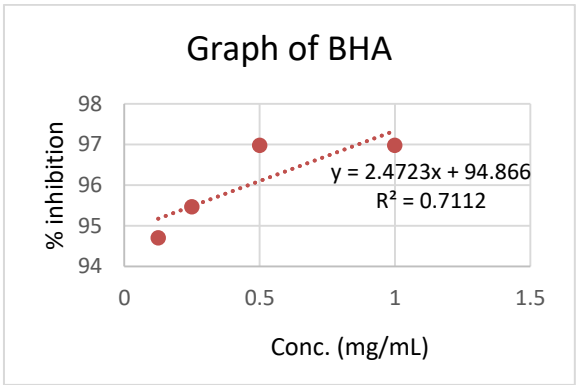

Fig. 1e: Butylated hydroxylanisole showing the IC_{50}.

3.3. Antimicrobial Activity of P. mildbraedii stem extracts

The potentially useful antimicrobials used in healthcare are being severely reduced by the emergence of multidrug resistance in bacterial strains. One factor could have been the long-term effects of the unchecked use and abuse of antimicrobial goods and agents. To combat multidrug-resistant organisms, natural compounds like plant extracts may be an option. The tested essential oil showed significant to moderate efficacy against the examined strains of bacteria and fungus. The antimicrobial screening of *P. mildbraedii stem extracts* shows how sensitive and resistant the tested microorganisms. *P. mildbraedii stem extracts* showed more sensitivity to antimicrobial activity than resistivity. The minimum inhibitory concentration of *P. mildbraedii stem extracts* inhibited the growth of *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *P. notatum* at 25 µg/mL and at 12.5 µg/mL the plant extracts inhibited the growth of *Penicillum notatum* resist *A. niger* and

Candida albicans (Usman and Osuji, 2007). Comparatively, *S. aureus* was resistant to all leaf extracts of *P. mildbraedii* tested earlier according to this investigation's findings about their antibacterial activity. *P. mildbraedii* stem extract has demonstrated nearly comparable bactericidal and bacteriostatic activity against both gram-positive and gram-negative bacteria after being evaluated by the MBC. Several microbe species, including *S. aureus, B. subtilis, P. aeruginosa, S. typhi* and *K. pneumoniae,* are reported in this study to be successfully treated with the extract as an antiseptic. The phytochemicals found in the *P. mildbraedii* stem extract may be the cause of the considerable antibacterial activity seen in this investigation.

Table 2: Inhibition of zone and sensitivity of Tested Microorganisms with *P. mildbraedii* Methanol stem extracts

Test organism	Sensitivity	Zone of inhibition (mm)
Klebsiella pneumoniae	S	27
Pseudomonas aeruguinosa	S	24
Staphylococcus aureus	S	22
Escherichia coli	R	0
Salmonella typhi	S	25
Bacillus subtilis	S	27
Aspergillus niger	R	0
Candida albicans	R	0
Penicillum notatum	S	24
Rhizopus spp.	R	0

Key: S = Sensitive, R = Resistance

Table 3: Minimum inhibition concentration (MIC) of *P. mildbraedii* Methanol stem extracts

Test organism	Concentration (µg/mL)				
	100	50	25	12.5	6.25
Klebsiella pneumoniae	-	-	-	0*	+
Pseudomonas aeruguinosa	-	-	0*	+	++
Staphylococcus aureus	-	-	0*	+	++
Escherichia coli	-	-	-	-	-
Salmonella typhi	-	-	0*	+	++
Bacillus subtilis	-	-	-	0*	+

Key: **-:** No turbidity (no growth), 0*: MIC, +: Turbid (light growth), ++: Moderate turbidity.

Table 4: Minimum bactericidal/fungicidal concentration (MBC/MFC) of *P. mildbraedii* Methanol stem extract

Test organism	Concentration (µg/mL)				
	100	50	25	12.5	6.25
Aspergillus niger	-	0*	+	++	++
Candida albicans	-	0*	+	++	++
Penicillum notatum	-	0*	+	++	++
Rhizopus spp.	-	-	-	-	-

Key: **-:** No turbidity (no growth), 0*: MIC, +: Turbid (light growth), ++: Moderate turbidity.

4. Conclusion

Phytochemical screening of *Pterocarpus mildbraedii* stem extracts has revealed the presence of several biologically active phytoconstituents, including flavonoids, alkaloids, tannins, phenol and steroids. And these phytoconstituents' presence in *P. mildbraedii* stem extracts is responsible for the significant antioxidant and antimicrobial activities at the tested concentrations. *P. mildbraedii* stem extract can serve as a potential natural source for pharmacological activities such as anti-inflammatory, antioxidant, and antimicrobial effects.

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