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Evaluation of the Phytochemistry, Antimicrobial and Antioxidant Efficacy of the Stem Bark Extracts of *Pterocarpusmildbreadii*

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Abstract: *In recent times there has been an upsurge in the search for new chemical agents to replace the conventional drugs, which have been characterized by drug resistance of microbes and the deleterious side effects associated with them. The use of any plant as medicine is attributed to its biological activities. Pterocarpusmildbreadii is a medicinal plant belonging to the family fabaceae, that abounds in Ghana, with its potentials yet to be explored. The present work aimed at screening the stem bark extracts of the variety of P. mildbreadii in Kumasi, Ghana, for their phytochemical constituents, antimicrobial and antioxidant activities aimed at finding future sources of antibiotics and antioxidants for pharmaceutical formulations. The antimicrobial efficacy was evaluated by the agar diffusion method, using Augmentin as standard; while antioxidant potential was assayed using 2, 2-diphenyl-1-picryl hydroxyl (DPPH) and hydrogen peroxide (H_2O_2). The phytochemical evaluation revealed the presence of phlobahannins, tannins, glycosides, saponins, alkaloids, terpenoids and steroids. Flavonoids and anthraquinone were absent in all the extracts. Test organisms for antimicrobial analysis include: Salmonella typhi, Staphylococcus aureus, Escherichia coli and Campylobacter. The extracts were found to be susceptible to all the tested organisms, with varying zones of inhibition. The zone of inhibition for the hexane extract range from 6 -19mm, that of ethyl acetate range from 9 – 20mm, while the ethanol extract range from 9 – 15mm. The extract showed concentration dependent antioxidant activity, with maximum % DPPH free radical scavenging activities of the hexane, ethyl acetate and ethanol extracts as; 86.16, 83.98 and 87.60 respectively as against that of the standard ascorbic acid which is 89. 55. For percentage H_2O_2 scavenging activity, the ethanol extract showed promise with the highest percentage of 93.03; performing better than the standard which is 85.93 %. Results obtained indicates pterorocarpusmildbreadii stem bark extracts have potentials as antibiotic and free radical oxidative inhibitor, suggesting continuous evaluation and isolation of active agents in the extracts.*

Keywords*: Pterocarpusmildbreadii, antioxidant activity, DPPH, antimicrobial activity, H_2O_2*

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I. Introduction

Plants have served as important sources of drugs since time immemorial in the traditional medicinal system. Most of the plants are used in form of crude extracts, infusions, tinctures or decoctions. According to WHO, over 80 percent of the population of the world depend on medicinal plants for their primary health care [1]. The significance of plants in medicine still remains very relevance with the current global tilting to obtain drugs that are plant-based, resulting in great attention being given to the medicinal value of herbal remedies for safety, potency and cost effectiveness[1,2].

In spite of the fact that human medicine has advanced greatly, infectious diseases have remained a major threat to public health. In developing country for instance, the impact of bacterial infection is great, most times leading to death; this is due to inaccessibility or unavailability of drugs, particularly in remote areas. To cap it all, wide spread drug resistance is another challenge that has to be dealt with [1,3]. Nature indeed is a reservoir of biological molecules that serve as cure for all human diseases or illnesses. Medicinal plants have since prehistoric days been the backbone of traditional medicine system. Scientific innovation and discoveries led to a proliferation of synthetic drugs of all kinds, but the joy of these discoveries was short-lived, because in its wake was discovered most of the synthetic drugs are accompanied by either drug resistance by microbes or other adverse or undesirable side effects. As a result, attention is being re-focused on traditional or herbal medicine. At present 75 % of the drugs in the market for treating infections and infectious ailments are sourced from plants or their analogues derivatives [4].

The plant *Pterocarpusmildbreadii* is highly valued in the traditional medicine system in Eastern and Western part of Nigeria, its leaves are consumed as a native delicacy rich in nutrients. The leaves are said to possess great potentials as; antimicronial agents [5], antioxidants [7,8], analgesic and treatment of respiratory

disorder [9].Amadi*et al*[10] discovered alkaloids, glycocides, saponins, tannins, terpenoids, flavonoids, phlobatanins, anthraquinones and steroids were found in varying amounts in P. Mildbreadii and other plants tested. Uchegu*et al*[11] carried out phytochemical analysis of *pterocarpusmildbreadii* leaves extract. The phytoconstituents in the ethanolic extract was investigated using GC-MS analysis. Seven phytochemicals were identified in the leaves extract, they include: fagasterol or lupeol constituting the bulk of the oil (42.04%), followed by oleic acid (20.38%), and palmitic acid (11.46%). Other constituents are: 1,2,3,4 Butanetetrol or Erythritol (5.73%), N, N Dimethyl-2-propyn-1- amine (4.46%), 1, 2-Benzenediol (7.64%), 4-Hydroxypiperidine (8.28%), and n-Hexadecanoic acid (11.46%). The presence of these compounds in the plant proved that the plant can be used as a potential food and drug.

With the great medicinal value derived from this plant, there is no assessment done on the biological potency of the stem bark in area of antimicrobial and antioxidant analysis, nor has anyone evaluated the potential of the plant variety in Kumasi, Ghana. The current research is aimed at examining the phytochemical as well as the antimicrobial and antioxidant potential of the stem bark of *Pterocarpusmildbreadii* in Kumasi, Ghana.

II. Materials And Methods

2.1 Sample Collection and Preparation

The fresh stem bark was collected in August, 2016 in Kumasi Ghana. The plant was identified as *Pterocarpusmildbreadii* in the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology, Kumasi. The voucher specimen, KNUST/HM1/2016/002 was deposited in the herbarium. The stem bark was sorted and cleaned, then shredded into smaller pieces using knife and air dried under shade for one week. The dried stem bark was then pulverized into powder. Powdered sample was then packed in air tight polyethylene bag and stored in preparation for extraction.

2.2 Extraction

The pulverized stem bark of *Pterocarpusmildbreadii* was extracted separately with hexane, ethyl acetate and 80 percent ethanol. 250g of stem bark powder was macerated in 1 liter of hexane [(250 x1) x3], covered and kept on a shaker for 24 hours at room temperature. Same procedure was repeated with ethyl acetate and 80 % ethanol. The extract was filtered with eight layers of muslin cloth and concentrated under reduced pressure at 40^{o}C using a rotary evaporator.

2.3 Phytochemical Analysis

The extracts were subjected to qualitative phytochemical analysis for metabolites such as flavonoids, saponnin, tannin, anthraquinones, Phlobactannins, steroids terpenoides and glycosides using standard procedures [12].

2.4 Antimicrobial assay

2.4.1 Test Microorganisms

The microorganisms used include: *Staphylococcus aureus, E. coli, S. typhi*and *campylobacter*. The microbes were obtained from the microbiology laboratory of the National Research Institute of Chemical Technology, Zaria.

2.4.2 Antimicrobial Activity

The method used by Mofio et al[13] was adopted. The extracts were dissolved in DMSO and used in varying concentrations ranging from 0.125 to 1mg /ml. Each concentration was pipetted into a sterile paper disc, the solvents were allowed to dry and the disc were then placed on the surfaces of petri dishes that were previously surface inoculated with the test microorganisms. This was followed by incubation of the plates at 37 ^{o}C for 24 hours. Augmentin was used as a standard. A measurement of the diameter of the zone of inhibition, gave a measure of the antimicrobial activity of the extracts. The experiment was done in triplicate.

2.5 Antioxidant Assay

2.5.1 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The capacity of the ethanolic extracts to scavenge free radicals was monitored according to the method described by Pajani*et al*[14]. The hydrogen atom or electron donating abilities of the extracts were measured from the bleaching of purple colour of the methanol solution of the stable 2, 2-diphenyl-1-picrylhydraxyl free radical. 1ml of ethanol extracts solution with varying concentration (20, 40, 60, and 80 µg/ml), was mixed with I ml of 0.1 mM DPPH in methanol solution. A corresponding blank sample and ascorbic acid (20-80 µg/ml) was used as reference standard. Reactions were incubated for 30minutes in the dark; the absorbance was measured at 517nm using Cecil UV/Visible 700 series spectrophotometer. The inhibition percent was calculated using the formula:

Inhibition % = Ac – As / Ac x 100

Where Ac is the absorbance of the control and As is the absorbance of the test sample. All tests were carried out in triplicate.

2.5.2 Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide scavenging activity was carried out according to the procedure outlined by Malu*et al*, [15]. This technique is based on the principle that there is a decrease in absorbance of hydrogen peroxide upon its oxidation. Stock solutions of both standard and sample were prepared with a concentration of 1000 µg/ml; there were then diluted to final concentrations of 20, 40, 60, and 80 µg / mL. A phosphate buffer solution (pH 7.4) was used to prepare a 40 mM solution of H_2O_2. 0.6 mL of the respective test and standard sample was added to 3.4 mL of hydrogen peroxide solution. The mixture was thoroughly mixed and incubated for 10 minutes at 37 Oc. A spectrophotometer (Cecil UV/Visible 7000 series) was used to measure the absorbance at wavelength 230 nm. Hydrogen peroxide solution was used as blank/control. All experiments were done in triplicate. The H_2O_2 scavenging activity was measured thus:

H_2O_2 scavenging % = Ac – As / Ac x 100; where Ac and As are the absorbance control and sample respectively.

III. Results

In the phytochemical analysis, all extracts tested positive for tannins, alkaloids, terpenoids, glycosides, steroids and phlobactannin. Flavonoids and anthraquinone were not found in all three extracts; only the hexane and ethanol extracts had saponnins (Table 1).

Table 1: phytochemical analysis of hexane, ethyl acetate and ethanol extracts of the stem bark of Pterocarpusmildbreadii

phytochemicals	P_M HEX	P_M ETOAC	P_M ETOH
Flavonoids	-	-	-
Tannins	++	++	++
Saponin	+	-	++
Alkaloids	++	+	++
Terpenoids	++	++	++
Anthraquinone	-	-	-
Glycosides	+	+	+
Steroids	++	+	++
Phlobactannin	++	+	++

Key: - not present; + present in low concentration; ++ present in high concentration.
P_M HEX (Hexane extract); P_M ETOAC (ethyl acetate extract); P_M ETOH (ethanol extract)

In the antimicrobial assay, *Pterocarpusmilbreadii*stem bark extracts showed varying degree of activity against all tested microbes (Table 2). The highest zone of inhibition was exhibited by the ethyl acetate on E. Coli (20mm), followed by hexane extract (19mm) on campylobacter. The ethanol extract shows promise (15mm) on both campylobacter and S. Typhi.

Table 2: Antimicrobial Activity of *Pterocarpusmildbreadii* Stem Bark

Test organism	P_m HEX extract (mm)	P_m ETOAC extract (mm)	P_m ETOH extract (mm)	Augmentin
Campylobater	19	9	15	20
S. Typhi	17	17	15	24
S. Aureou	6	14	9	22
E. Coli	11	20	11	22

In the antioxidant assays in both DPPH and H_2O_2, the radical scavenging activities of the extracts were found to be concentration dependent (Table 3 & 4).

Table 3: DPPH Free Radical Scavenging Activity of *Pterocarpusmildbreadii*

Conc. µg/ml	PS_{HEX}	PS_{ETOAC}	PS_{ETOH}	Asc.Acid
20	70.74±0.769	73.56± 0.531	60.03 ± 0.154	78.37 ±0.462
40	83.20±0.363	74.67± 0.497	72.89 ± 0.354	85.86± 1.860
60	84.31±0.381	76.62± 0.454	82.87 ± 0.308	87.91 ±0.254
80	86.16±0.407	83.98± 1.225	87.60± 0.254	89.35 ± 1.256

Table 4: peroxide scavenging activity of *Pterocarpusmildbreadii*

Conc. µg/ml	PM_{HEX}	PM_{ETOAC}	PM_{ETOH}	Asc.Acid

20	22.35±2.894	23.30± 1.142	62.62 ± 0.601	58.31 ±1.445
40	46.91±1.765	31.98± 2.525	63.87 ± 1.754	61.95± 1.168
60	53.66±1.924	38.72± 2.182	69.75 ± 1.693	68.21 ±0.867
80	60.50±1.303	45.47± 2.851	93.06 ± 2.005	85.93 ± 6.092

With ethanol extract showing great promise in scavenging both the DPPH free radical and H_2H_2 radicals, with 87.60 and 93.06 respectively. The hexane extract also shows great promise in scavenging the DPPH free radical with 86.18 percentage scavenging activity.

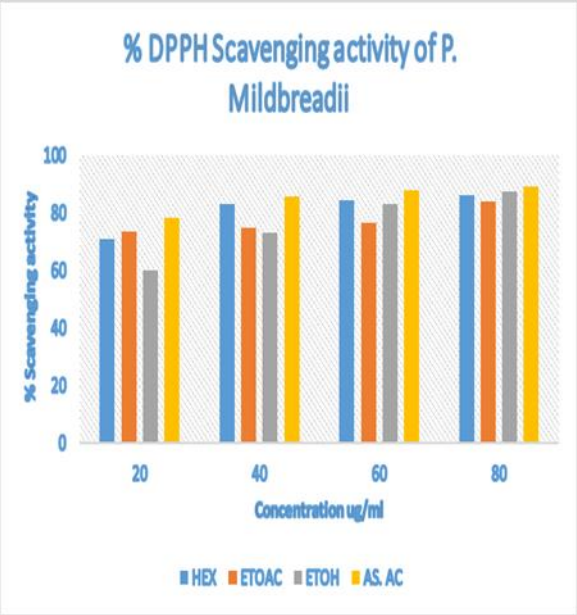

Fig. 1: % DPPH Scavenging Activity

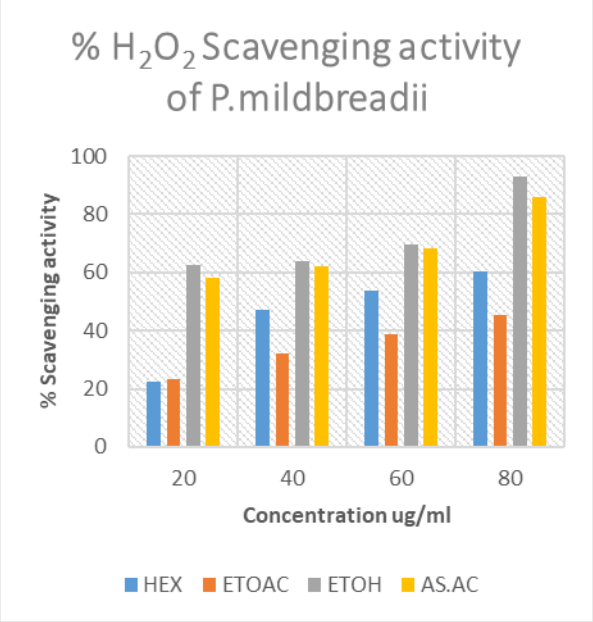

Fig 2: % H_2O_2 Scavenging activity

Key: HEX (hexane extract); ETOAC (ethyl acetate extract); ETOH (ethanol extract).

IV. Discussion

Anthraquinone was discovered to be absent in all the extracts, which is consistent with the study of the bark extracts of plant of same genus by Gabriel and Onigbanjo[16]. The phytochemical contents were also found to be consistent with the works on plant of same genus by Ogbonna and Idumah[17], except with respect to flavonoids. Alkaloids are said to possess antimicrobial properties [1], analgesic and reduce headaches and fever [18].Saponins and terpenes on the other hand are known to inhibit the growth of bacteria and render protection to plants against bacteria and fungi[1,19,20]. Tannin inactivate microbial adhesions, enzymes and also complex with polysaccharides [21]. The antimicrobial activity of *Pterocarpusmildbreadii* may be due to these secondary metabolites acting either individually or working together in synergy to exhibit antimicrobial activity. The phytochemicals are responsible for a wide range of biological activities as a consequence of their antioxidant activity [22]. The result gives credence to the use of the plant in treatment of diarrhea, justifying its usage in traditional medicine.

For the antimicrobial assay, E. Coli was highly susceptible to the ethyl acetate extract with inhibition zone of 20mm, campylobacter had high susceptibility for the hexane extract and ethanol extract with an inhibition zone of 19mm and 15mm respectively; S. Typhi showed high susceptibility for the hexane and ethyl acetate extracts with zone of inhibition of 17mm each. The high sensitivity of the extracts against some of the microorganism tested tallies with works of Gabriel and Onigbanjo[16] and further justifies the use of the plants of this genus in traditional medicine.

In the DPPH free radical scavenging, extracts reacted with the DPPH to discolour the deep violet colour, with the intensity of discolouration indicating the free radical scavenging potential of the extract. The DPPH method is based on the reduction of DPPH in alcoholic solution in the presence of an antioxidant hydrogen donating specie leading to the formation of a non-radical or stable form DPPH-H in the reaction. The scavenging effect of *Pterocarpusmildbreadii* stem bark extracts increase with increase in concentration, being

consistent works of Anuj*et al*, [23] and Feki*et al*, [24]. At 80 µg/ mL the percent scavenging activity was found to be 86.16, 83.98 and 87.60 for hexane, ethyl acetate and ethanol extracts respectively; while that of the standard was 89.55. Antioxidant activity may be due to the presence of tannins [25,26]. For the hydrogen peroxide scavenging activity, only the ethanol extract showed great promise. The ethanol extract gave the highest value for percentage H_2O_2 scavenging activity,93.06 which is higher than that of the standard ascorbic acid, 85.93. It is well known that tannins are natural antioxidants (Priyanka *et al*, 2013; Akaniro-Ejim*et al*, 2018). *Pterocarpusmildbreadii* showed a dose dependent inhibition of hydrogen peroxide, and the ethanol extract showed better scavenging activity than the standard ascorbic acid.

V. Conclusion

The phytochemicals present justify the use of P.mildbreadii in the African tradition medicine system. Alkaloids, terpenoides and steroids, have activity against bacteria, justifying plants usage in diarrheal control in Trado-medicine. Alkaloids are analgesic and reduce headaches and fever, justifying plants usage for fever and pains. The presence of alkaloids, tannins, saponins, terpenoides and phlobantannins in the stem bark of *Pterocarpusmildbreadii* has given scientific credence to the use of the plant in traditional medicine system.

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Competing Interest: Authors declare there is no conflict of interest.

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