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Phytochemical Analysis and Biological Assay of the Stem Bark of *Pterocarpus Santalinoides* in Kumasi, Ghana.

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Abstract: *The use of any plant as medicine is attributed to the presence of phytochemicals which are responsible for the biological activities. Pterocarpussantalinoides is a medicinal plant belonging to the family fabaceae. The stem bark extracts of the plant were evaluated for their phytochemical constituents; tested for antimicrobial activity (with Augmentin as standard) by the agar diffusion method and evaluated for their antioxidant potential by DPPH free radical scavenging and H_2O_2 scavenging activities. Results of the phytochemical analysis revealed the presence of flavonoids, phlobatannins, tannins, glycoside, saponins, alkaloids and terpenoids in all the three extracts (ethanol, hexane, and ethyl acetate extracts); while the hexane and ethyl acetate extracts contain in addition to these, steroids. Anthraquinone was however only present in the hexane extract. Test organisms for antidiarrheal activities include Salmonella typhi, Escherichia coli, Campylobacter, and Staphylococcus aureus. The extracts showed varying degree of inhibitory activity against tested organisms. The zone of inhibition for the hexane extract range from 7 -19mm, that of ethyl acetate extract range from 9 – 13mm, while that of ethanol extract range from 9.5 – 15mm. The antioxidant activity was found to be concentration dependent. For DPPH radical scavenging, hexane extract had the highest percent scavenging value (86.16 %) comparable with that of the standard ascorbic acid (89.35%). H_2O_2 scavenging activity of the extracts was also promising, ethanol extract had the highest value (85.55%) which was comparable to that of the standard ascorbic acid (85.93 %). The results show that the traditional use of the plant Pterocarpus santalinoides is justified especially in the treatment of diarrhea. The variety pterocarpussantalinoides in Kumasi is a promising source of antimicrobials and antioxidants due to its rich phytochemical content.*

Keywords*: ethanolic extract, antioxidant activity, DPPH, antimicrobial activity, H_2O_2*

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I. Introduction

Africa has a rich history in traditional healing, especially in the use of the diverse medicinal plants available. These plants are used to treat various health conditions ranging from cough, diarrhea, headaches, fever, skin diseases, stomach upset to mention but a few. However, scientific research on the biological efficacy of most of these plants is lacking. The presence of antimicrobial compounds in plants serves as a useful area in the development of natural products that come handy in replacing the antibiotics against antibiotic resistant pathogens, and serves as a foundation for new antimicrobials.

Man has grappled with numerous diseases since time immemorial, it is now widely known that free radicals are implicated as being responsible for most human degenerative diseases; as most of the diseases have been traced to uncontrollable production of free radicals, which accumulates in the body, and causes oxidative stress[1], this leads to diseases when its abundance becomes unbalanced. Artificial antioxidants such as butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone (TBH), butylatedhydroxyl anisole (BHA) and gallic acid esters are being implicated in negative health effects, thus, there is a strict restriction placed on their application [2]. There is now a trend to substitute them with naturally occurring antioxidants [3].

Herbal remedies play an important role in maintaining the health of man. It is common knowledge today that the majority of the world uses herbal remedies; this is also approved by world health organization as safe alternative to synthetic drugs due to the fact that they have stood the test of time with regards to safety, efficacy, cultural acceptance and minimal adverse effect. According to world health organization (WHO), about 21,000 plants have been used for medicinal purposes and 80% of the world relies on traditional medicine [4]. The medicinal potentials of these plants lie in the presence of some secondary metabolites, collectively called phytochemicals. These phytochemicals have great potentials as herbal medicines or as precursors for modern medicines.

Generally, plants form the global basis of traditional medicine system for thousands of years, and have continued to provide man with new health remedies. Such remedies usually dispensed in the form of crude drugs such as teas, tinctures or other herbal formulations now serves as basis for novel drug discovery. Historically, the plant-based knowledge was handed down from one generation to another in various parts of the world. Till date, plants still provide new chemical entities as lead molecules for the development of drugs against various diseases. Thus, plant-based drug discovery will continue to be an important source of new drug leads.

Pterocarpussantalinoides commonly called the red sandal wood in English and *Uturukpa* in Eastern Nigeria, is a tropical/sub-tropical tree found in Africa in countries such as; Cameroon, Nigeria, Ghana, Sierra Leone, and Equatorial Guinea [5]. *Perocarpussantalinoides* has been used traditional in many African countries for the treatment of many diseases like diarrhea, dysentery, aches and pains, management of fever and blood pressure. Few scientific research works have also been carried out to evaluate the traditional claims on its efficacy. Some of the research findings are: The leaves of *pterocarpussantalinoides* increases the level of haemoglobin, packed cell volume and platelets[6;] relieve pyrexia [7,8,9], The stem bark was said to possess antimicrobial activity [10]; The bark extracts are used in the treatment of diabetes, cough and sore belly [11,12,13].

Previous literature shows phytochemical study and antimicrobial study on the leaves of Pterocarpussantalinoides**,** but there is very little report of the phytochemical and antimicrobial activity of the stem bark, there is no report on the antioxidant potential of the stem bark. Also the variety of the plant in Ghana is completely unexplored. Thus, the aim of this research is to evaluate the antimicrobial and antioxidant potential of the stem bark extracts of *Pterocarpussantalinoides* found in Kumasi, Ghana.

II. Materials and Methods

Sample Collection and Preparation

The fresh stem bark was collected in August 2016 in Kumasi Ghana. The plant was identified as *Pterocarpussantalinoides* 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 using an electric grinder. Powdered sample was subjected to hexane, ethyl acetate and hydro-ethanol extraction.

Extraction

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

Phytochemical Analysis

Qualitative phytochemical analysis was carried using standard procedures.

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.

Antimicrobial Activity

The method used by Mofio*et al*[14] was adopted. The extracts were dissolved in DMSO and used in varying concentrations ranging from 0.125 to 1 mg/ 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.

Antioxidant Assay

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* [15]. 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 517 nm 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. Extract concentration causing 50% inhibition (IC_{50}) was obtained from graph. All tests were carried out in triplicate.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide scavenging activity was carried out according to the procedure outlined by Malu*et al*[16]. 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; these 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 ^{O}C. 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

The search for phyto-constituents possessing antimicrobial and antioxidant potentials are presently on the increase due to their therapeutic potentials in treating various infectious and chronic diseases.**Table no. 1** shows the phytochemicals present in the three extracts. The hexane extract (P_S HEX) contains: flavonoids, tannins, saponins, alkaloids, terpenoids, anthraquinone, glycosides, steroids andPhlobactannin. The ethyl acetate extract (P_S ETOAC) contains all the phytochemicals in hexane extract except anthraquinone; while the ethanol extract (P_S ETOH) contains all the phytochemicals in hexane extract except anthraquinone and steroids.

Table no. 1: shows the phytochemical constituents of stem bark extracts of Pterocarpussantalinoides

Phytochemicals	P_S HEX	P_S ETOAC	P_S ETOH
Flavonoids	+	+	++
Tannins	+	+	+
Saponin	++	++	+
Alkaloids	++	+	++
Terpenoids	++	++	++
Anthraquinone	+	-	-
Glycosides	+	+	+
Steroids	++	++	-
Phlobactannin	++	++	+

Table no. 2 shows zone of inhibition obtained in antimicrobial assay of hexane, ethyl acetate and ethanol extracts of P. santalinoides. All extracts were susceptible to test microbes at varying degrees, with hexane extract having zone of inhibition that range from 7-19mm, ethyl acetate extract has zone of inhibition ranging from 9-13mm; while ethanol extract has zone of inhibition that range from 11-15mm.

Table no. 2: shows the Antimicrobial Activity (zone of inhibition) of stem bark extracts of P.santalinoides

Test organism	P_s HEX extract (mm)	P_S ETOAC extract (mm)	P_S ETOH extract (mm)	Augmentin
Campylobater	16	9	13	20
S. Typhi	12	13	15	24
S. Aureou	7	13	9.5	22
E. Coli	19	11	11	22

Key: P_S HEX (Hexane extract); P_S ETOAC (ethyl acetate extract); P_S ETOH (ethanol extract)

Table no 3, shows percentage DPPH radical scavenging activity of hexane, ethyl acetate and ethanol extracts as compared with that of the standard ascorbic acid. All the extracts show a concentration dependent radical scavenging hehaviour. At 80 µg/ml, percentage DPPH scavenging activity were found to be 86.16, 74.10, 71.28 and 89.35 for hexane, ethyl acetate, ethanol extracts and ascorbic acid respectively.

Table NO. 3: DPPH RadicalScavenging Activity of ExtractS of *P. santalinoides* Stem Bark

Conc. µg/ml	PS_{HEX}	PS_{ETOAC}	PS_{ETOH}	Asc.Acid
20	70.74±0.769	51.19± 0.407	43.70 ± 0.533	78.37 ±0.462
40	83.20±0.824	64,72± 0.419	54.55 ± 0.648	85.86± 1.860
60	84.38±0.381	79.88± 0.461	65.90 ± 0.454	87.91 ±0.254
80	86.16±0.407	74.10± 0.407	71.28 ± 0.254	89.35 ± 1.256

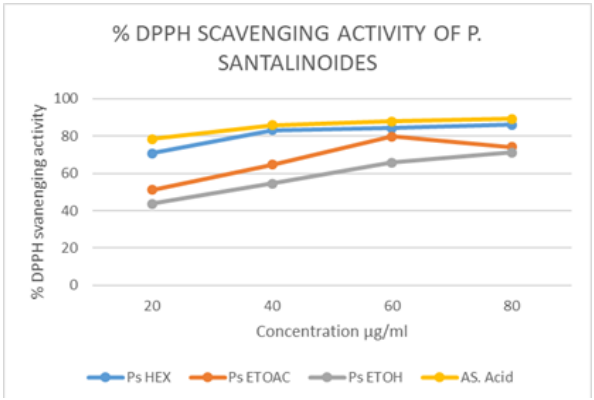
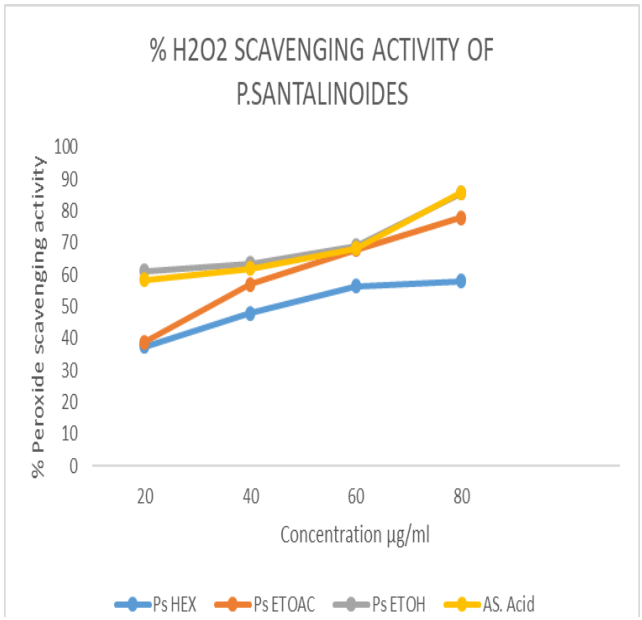


Table no. 4 shows H_2O_2 scavenging activity of the extracts and the standard, they all increase with increase in concentration. Percentage peroxide scavenging activity at concentration of 80 µg/ml were 58.00, 78.03,85.55 and 85.95 for hexane, ethyl acetate, ethanol and the standard respectively.

Table no. 4: H_2O_2 Scavenging Activity of Extracts of *P. santalinoides* Stem Bark

Conc. µg/ml	PS_{HEX}	PS_{ETOAC}	PS_{ETOH}	Asc.Acid
20	37.37±2.622	38.82± 1.303	61.09 ± 1.483	58.31 ±1.445
40	48.±1.742	56.93± 10.04	63.49 ± 0.88	61.95± 1.168
60	56.47±3.183	67.88± 1.425	68.98 ± 1.483	68.21 ±0.867
80	58.00±8.457	78.03± 4.938	85.55 ± 5.685	85.93 ± 6.092



IV. Discussion

Results of this study showed that the extracts of the stem bark of *Pterocarpussantalinoides*contain important phytochemicals such as: flavonoids, phlobatannins, glycosides, tannins, saponins, steroid, alkaloids and terpenoids (Table 1). Phytochemical content agrees with the research findings of Ogbonna and Idumah[17]; and that of Eze et al [10]. Flavonoids have been found to possess antimicrobial, anti-inflammatary and antioxidant activities[18, 19]. Certain biological activities are said to be due to the presence of flavonoids; these includes but not limited to: antiviral, antibacterial, anti-inflammatory, antidiarrheal, antitumor and also in the treatment of neurogenerative illnesses, they are also known to inhibit lipid peroxidation. These effects are exerted as antioxidants, chelators of cations, scavengers of free radical [18,20,21]. The presence of polyphenols such as flavonoids and tannins may be the scientific bases for the use of the plant *Pterocarpussantalinoides* in the treatment of diarrhea and skin infections.

The results of the antimicrobial activity show that the extracts have varying degree of inhibitory activities against all tested microorganisms, agreeing with the work of Eze et al [10] on the stem bark of P. Santalinoides. The inhibitory activities were however, lower than that of the standard Augmentin. E. Coli and Campylobacter showed high sensitivity for the hexane extract, with an inhibition zone of 19mm and 16mm respectively. *S.typhi* and Campylobacter showed high susceptibility to the ethanol extract with an inhibition zone of 15 mm and 13mm respectively. E. Coli and S. Typhi also showed high sensitivity for the ethyl acetate extract with 13mm zone of inhibition. The zone of inhibition of the hexane extract ranges from 7mm – 19mm, that of ethyl acetate range from 9mm – 13mm, while that of the ethanol extract range from 9.5mm – 15mm, Augmentin gave zones of inhibition ranging from 20mm – 24mm; implying Augmentin is more active than the stem bark extracts. The presence of flavonoids in all the extracts is responsible for the antimicrobial activity. Sourcing for antimicrobial agent from natural sources as done in this research, contributes to the development of effective screening systems for lead compounds [22]. All organisms in this research were selected based on their link or relationship with traditional medicinal use of the plant in question. Specifically, three of the organisms are diarrhea causing organisms (*campylobacter, S. typhi*and *E. coli*) while one is linked to skin infections (S. aureus).

DPPH usually used as a reagent to evaluate free radical scavenging activity of antioxidants, is an unstable free radical which can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radical scavenging assay is commonly used for rapid evaluation of antioxidant efficacy of a compound [23]. The percentage scavenging activity of DPPH radical by P. santalinoides stem bark extracts is given in Table no. 3. The DPPH radical scavenging activity of the extracts of the stem bark of *P. santailinoides*showed a concentration dependent activity. the hexane extract had percent scavenging activity comparable with that of the standard ascorbic acid (86.16 and 89.55 respectively). Data was extrapolated to obtain IC_{50} as 5.82 and 3.77 for hexane extract and ascorbic acid respectively.

This concentration dependence in scavenging property of *P.santalinoides*, reflects its high antioxidant efficacy and justifies its use in traditional medicine. As the concentration of the extract increases, the percentage inhibition of the extract on DPPH becomes quite close to that of the standard compound used. This explains why the plant is extensively used in the traditional medicine system. The high antioxidant capacity could be due to the presence of flavonoids and tannins [24].

Hydrogen peroxide H_2O_2, a non-radical reactive oxygen species (ROS) is biologically very important and can influence cellular processes. Among the ROS, hydrogen peroxide is by itself nontoxic; but it can be converted to toxic radical like OH by either Fenton reaction or hypochlorous acid by enzyme myeloperoxidase [25]. Uncontrolled generation of ROS results in redox imbalance and oxidative stress which are harmful. The H_2O_2 scavenging activity of *P. santalinoides* stem bark was evaluated and compared to that of ascorbic acid. The percentage inhibition of the extracts as well as that of ascorbic acid (the standard) at various concentrations (20 – 80 µg /mL) were calculated and presented in Table no 4. Of the three extracts, Ethanol extract had the highest percentage scavenging activity of 85.55 which is similar to that of the standard ascorbic acid at 85.93, this shows that polyphenol compounds are present in the ethanol extracts of *Pterocarpussantalinoides*, which agrees with the phytochemical screening data. It is well known that flavonoids and tannins are natural antioxidants [26,27]. Tannins are astringent in nature and are used in treating intestinal disorders such as diarrhea and dysentery,

V. Conclusion

The stem bark of *Pterocarpussantalinoides* in Kumasi has been found to be rich in polyphenolic compounds such as flavonoids and tannins as well as other metabolites, justifying the usage of this plant in Africa and other parts of the world for the treatment of diarrhea, dysentery and other gastrointestinal disorders in the traditional medicine system. The stem bark of *P.santalinoides*from Kumasi, Ghana; would offer protection against oxidative damage to cells.

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