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REVIEW ARTICLE

THE PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF PTEROCARPUS SANTALINOIDES PLANTS

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ABSTRACT

Sequel to the use of the plant parts of P. santalinodes (Nturu Ukpa) in traditional medicine the phytochemical and antimicrobial analysis of the leaves and stem were undertaken. Qualitative and quantitative evaluation of the phytochemical was made using standards methods. The microbiological analysis of the ethanol and methanol extract of the samples were performed on some test organisms *Escherichia. coli, Staphylococcus. aureus, Klebsiella. pneumonia, Pseudomonas. aeruginosa, Bacillus. subtilis, Aspergillus. niger, Aspergillus. flavus, Trichophyton. rubrum and Microsporium. gypseum*, using agar diffusion method for the screening, nutrient agar and potatoe dextrose agar were used respectively for the invitro antimicrobial activities. The results shows that, the plant part is rich in bioactive substances such as alkaloid (2.64±0.01), flavonoid (2.0±0.00), tannin (1.52±0.01), Saponnin (2.5±0.02), terpens (2.6±0.01), cardiac glycoside (2.5±0.00) and Steroid (1.82±0.01). The antimicrobial analysis shows that ethanol extract of the leaves had more inhibition against S.aureus,E.coli,B. subtilis, K. pneumonia and P. aeruginosa, than the methanol extract of the stem bark. The antifungal activities of the leaves and the stem bark showed a higher inhibition on *A.niger, A.flavus, T.rubrum* and *M.gypseum* on 40mg/ml concentration of the extract. The minimum inhibitory concentration (MIC) evaluated on the ethanol and methanol extract using a two-fold serial dilution showed little zone of inhibition ranging from (1-3mm) in diameter. The anti nutrient composition presents are in negligible amount. These results indicate that the plant leaves and the stem bark have potential medicinal uses, and could protect the body against some common microorganisms.

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INTRODUCTION

Plants have formed the basis of traditional medicine system which has been used for thousands of years. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicine, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat or to diagnose and prevent illness or maintain well being (WHO, 2013). In developing countries where orthodox medicine are quite expensive, traditional medicine is widely practiced thus screening for antimicrobial active compound from ethno-medical plants, is vital so as to ascertain genuine active plants and active compounds. Ultimately, these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians (Ama, 2010). In Nigeria *Pterocarpus santalinoides* is used as food or medicine.

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The tender leaves are used as vegetable in soup preparing, while the stem bark is used in making pepper soup. The plants are used in treating rheumatism, diarrhea, dysentery, cough, ashma, diabetes, malaria, elephantiasis, cold and others. (Okwu and Ekeke, 2006). The use of the leaves in treating skin disease such as eczema, candidiasis, and acne have been reported (Adetunji, 2009). The use of the concoction made from its root in treating asthmatic patients have also been reported (Igoli, 2005). It is also used in treating diarrhea which a major cause of death as it has a proven anti enteropoling activity in traditional medicine (Ogbe *et al*; 2006). The anti-malaria activity has been reported in treating infertility in females (Alexis *et al*; 2006). The stem bark extracts is also used in treatment of cough and diabetes. The leaves used in veterine medicine to reduce abdominal pains in goat and also menses in female (Igoli *et al*; 2005, Ama, 2010). This study is to investigate the qualitative and quantitative phytochemical compositions, antimicrobial and its nutritional /mineral analysis of the *Pterocarpus santalinoides* parts in order to determine their medicinal and nutritional value.

Thus

- To determine the phytochemical composition of the plant parts (leave and stem bark)
- To study the antimicrobial activities of the plant extracts
- To determine inhibitory concentration (MIC) of the extract on organisms.

MATERIALS AND METHODS

Sterilization of Materials

The sterilization of glass wares was done using the hot air oven at 140^0c for 3 hours after washed with water, detergent and detol. The working tables were also swabbed with 75% ethanol before and after the experiment.

Sterilization of Culture Media

The culture media used were nutrient agar, P.D.A. and malt extract agar was prepared according to the manufacturer's description and was melted in the autoclave at the temperature of 121^0c for 15minutes

Plant Extraction

The *Pterocarpus santalinoides* plant was collected with the aid of a sterile axe at Okigwe Local Government Area of Imo State, southern part of Nigeria, identification was done at the National Horticulture Research Institute in Imo State (NIHORT) by a taxonomist. The stem bark and leaves were taken for analysis. The collected plant parts was washed with sterile water and they were allowed to air dry for 10 days. The sample which ground into powder using an electric blender. The powdered samples were soaked with ethanol (70%) and methanol (80%) for 5 days and then filtered using whatmann number 1 filter paper.

Ethanolextraction

Each 20g of the leaf and stem bark were soaked in a flask containing 200ml of 70% ethanol. The mixtures were agitated and left to stand. The mixtures were filtered after 2 days with whatmann number 1 filter paper. (Ogbulie *et al.,* 2007)

Methanol Extraction

Each 20g of the leave and stem bark were dissolved in 200ml of methanol, and was allowed to stand and filtered after 5 days with whatmann filter paper. All the extracts were stored in the refrigerator at 4^0c for 24 hrs.

Qualitative Phytochemical Screening

The methods described by Akerele *et al.* (2001) were used to test for the presence of Saponins, tannins, glycosides, flavonoids, terpenes, steroids and Alkaloids, Lieberman Burchad reaction as described by Akinpelu *et al.* (2009) was used for steroids, while the Salkowski test was used for the presence of glycosides.

Testing for Saponinns

Each extracts 0.5g was mixed with10ml water in test tube, foaming which persisted on warming, and was taken as an evidence for the presence of Saponinn.

Testing for Tannins

Each extracts (0.5g) was mixed with 10ml of distilled water, a few drop of 1% lead acetate was added, a yellow precipitate is formed, indicating the presence of tannin (Sofoware 2006).

Testing for Cardiac Glycoside

Exactly 0.5g of the extracts was dissolved in 2ml of chloroform. Tetraoxosulphate (vi) acid (H_2SO_4) was carefully added to form a lower layer. A reddish brown colour at the interface indicate the presence of a steroidal ring that is a gylycone portion of the cardiac glycosides.

Testing for Flavonoids

Each extract 0.5g was dissolved in1ml of dil. Sodium hydroxide, and was added. An intense yellow colour was produced from the extract solution which became colourless on addition of a few drops of dilute acid, indicated the presence of flavonoids

Testing for Terpenes

Exactly 0.5g of the extract was dissolved in (3ml) of the plant extract and the mixture was filtered. To the filtrate, 10 drops of acetic anhydride and drops of concentrated H_2So_4 were added. A pink colour at the interface indicated preliminary positive test for terpenes

Testing for Alkaloids

Exactly 0.5g of the extract was stirred with 5ml of 1% Hcl on a steam bath. The solution obtained was filtered and (1ml) of the filtrate was treated with a few drops of mayor's reagent. The turbidity of the filtrate on addition of mayor's reagent showed an evidence of the presence of Alkaloids in the extracts.

Testing for Steroids

Exactly 0.5g of the extract was dissolved in 2ml of acetic anhydride and then a drop of concentrated H_2SO_4. The mixture was steamed for 1 hour and neutralized by the addition of chloroform. The presence of a blue colour indicates the presence of steroid.

Quantitative Phytochemical Analysis

The methods described by Akerele *et al.* (2001) were used to test for the presence of Saponins, tannins, glycosides, flavonoids, terpenes, steroids and Alkaloids. Lieberman Burchad reaction as described by Akinpelu *et al.* (2009) was used for steroids, while the Salkowski test was used for the presence of glycosides

Determination of Alkaloid

About 1g of the leaf and stem bark sample were weighed out and macerated with 20ml of 2% H_2SO_4in ethanol (1:1). The mixture was filtered and 1ml of the filtrate was pipetted into test tubes. 5ml of 60% sulphuric acid and 5ml of 0.5%formaldehyde in 60% H_2SO_4 were added and the content of the test tube were mixed properly. The mixture was allowed to stand for 3hrs and absorbance was measured at 568nm.

Determination of Tannin

About 1g of the sample was weighed out and macerated with 50mls of distilled water. The mixture was filtered and 1ml of the filtrate was pipetted into test tubes and 2ml of the saturated picric acid was added. Absorbance was measured at 530nm.

Determination of Glycosides

About 1g of the samples was weighed out and 2.5mls of 15% lead acetate was added and the mixture was filtered. 2mls of chloroform was added to the filtrate and the mixture was shaken vigorously. The lower layer was collected and evaporated to dryness. 3mls of glacial acetic acid was added and 0.1ml of 5% ferric chloride and 0.25mls concentrated H_2SO_4 were added and the mixture was shaken. The mixture was then left to stand in the dark for 2hrs and absorbance was measured at 530nm.

Determination of Flavonoid

About 1g of each of the samples were measured out and macerated with 20ml of ethyl alcohol. The mixture was filtered, 5mls of the filtrate was measured into the test tubes. To each test tubes contain the filtrate, 5mls of dilute ammonia was added and the mixture was shaken. The supernatant was collected and absorbance was measured at 490nm.

Determination of Saponin

About 1g each of the samples was weighed out and macerated with 10ml of petroleum ether. The supernatant was decanted into a beaker and another 10mls of petroleum ether was added. The supernatant was decanted and mixed with the first. The mixture was evaporated to dryness and 6mls of ethanol was added. 2mls of the mixture was pipetted into a test tube. 2mls of colour reagent was added and the mixture was allowed to stand for 30minutes, then the absorbance was read at 550nm.

Determination of Steroid

About 1g each of the sample was weighed out and macerated with 20ml of ethanol. The mixtures were filtered and 2ml each of the filtrates were pipetted into test tubes. Then 2mls of yellow colour reagent was added into the test tubes and they were allowed to stand for 30minutes and the absorbance was measured at 550nm.

Determination of Terpenes

Exactly 1g of the sample was weighed out and macerated with 20ml of distilled water. The mixture was filtered and 2ml of the filtrate was pipetted into test tubes and 3mls of saturated hydrochloric acid was added. The absorbance was measured at 550nm.

Isolation/Identification of Bacterial Isolates

The bacterial test organisms used were isolated from the stock culture bought with code numbers A_1B, A_2B, A_3B, A_4B, A_5B in the microbiology unit of the General Hospital Okigwe and were further identified based on their morphology, microscopic appearance (gram reactions) and biochemical reactions at Department of Microbiology, Abia State University Uturu.

Isolation/Identification of Fungal Isolates

The microorganisms used for the screening include *Aspergillus niger. Aspergillus flavus, Trichophyton rubrum and microsporum gypseum.* The moulds (*A.niger* and *A. flavus)* were standard and packaged organisms obtained from mycology unit of General hospital Okigwe, Imo state. The dermatophytes namely *Microsporum gypseum and Trichophyton rubrum* were isolated. The organisms were obtained with consent from infected pupils in Okigwe. The pupils were randomly selected and physically screened for ringworm on the scalp *(Tinea capitis) and (Tinea corporis).* The infected portion of the scalp was first sterilized with methylated spirit and then scraped unto a sterile study with the aid of a sterile surgical blade. The organisms were cultured in potatoe dextrose agar (PDA) medium incorporated with 500 mg of chloramphenicol to inhibit the growth of any bacteria contaminant. Sub-cultures were made to obtain isolates which were identified through microscopy as pure colonies of *M. gypseum and T. rubrum* with the aid of their spores and hyphae.

Gram's Staining/Microscopy

This staining was used to differentiate bacterial cells into oxidase and catalase cells based on the colour reaction exhibited by the bacteria when they were treated with the dye

Determination of Antibacteria Properties of The Extracts

The isolates were tested for antibacterial susceptibility using the ethanol (70%) and methanol (80%) extracts of *Pterocarpus santalinoides* with chloramphenicol used as the control. The agar well diffusion method was employed. A loopfull of the isolated organisms were inoculated into a bijox bottle containing 5ml of peptone and then incubated for 3hrs to resuscitate the organisms. A solid nutrient agar plate was seeded with 1ml of the inoculum of the test, bacterial isolates. The plates were swirled, allowing the inoculum to spread on the surface of the agar, and the excess was discarded in a disinfectant jar. The plates were dried in the incubator for 15minutes at 37^0c with the aid of the sterile standard cork borer (6mm), wells were bored at equal distance around the plates. 0.2ml of each prepared concentration of the extracts was aseptically introduced onto the wells. The plates were allowed on bench for 40minutes, for pre-diffusion and then incubated at 37^{0c} over night. The resulting zones of inhibitions` were measured using a ruler calibrated in millimeters. The average of the reading was taken to be the

zone of inhibition of the bacterial isolates in question at that particular concentration.

Determination of Anti-Fungal Properties of the Extracts

The antifungal susceptibility test was carried out, and the agar plug method was used .A sterile clean cork borer was used to create a hole at the centre of the PDA plate. Another hole was created on the PDA plate containing the organism thereby transferring the organism into an already prepared plate thus filling the hole (plug) created earlier. A paper disk soaked with the different concentrations of the extracts was placed at an equal distance around the fungi plug. A nystatin drug was used as a control. The plates were incubated at 28^0c. Zone inhibition around the plug were observed and measured.

Determination of minimum Inhibitory Concentration (MIC)

The MIC of the ethanol and methanol of the leaf and stem bark were determined as described by Akinpelu and Kolawole (2004). Two fold- serial dilutions of the plant extracts were prepared to obtain 0.1 – 100mg/ml from which 2mls aliquots was taken and added to 18ml of pre-sterilized molten nutrient agar at a temperature of 40^0C. The media were then poured into sterile Petri dishes and allowed to solidify. The surfaces of the media were allowed to dry before streaking with 18h old cultures of the test organism. The plates were later incubated in an incubator at 37^0C for up to 72hrs.after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented bacterial growth.

Determination of Antibacteria Properties of The Extracts

The isolates were tested for antibacterial susceptibility using the ethanol (70%) and methanol (80%) extracts of *Pterocarpus santalinoides* with chloramphenicol used as the control. The agar well diffusion method was employed. A loopfull of the isolated organisms were inoculated into a bijox bottle containing 5ml of peptone and then incubated for 3hrs to resuscitate the organisms. A solid nutrient agar plate was seeded with 1ml of the inoculum of the test, bacterial isolates. The plates were swirled, allowing the inoculum to spread on the surface of the agar, and the excess was discarded in a disinfectant jar. The plates were dried in the incubator for 15minutes at 37^0c with the aid of the sterile standard cork borer (6mm), wells were bored at equal distance around the plates. 0.2ml of each prepared concentration of the extracts was aseptically introduced onto the wells. The plates were allowed on bench for 40minutes, for pre-diffusion and then incubated at 37^{0c} over night. The resulting zones of inhibitions` were measured using a ruler calibrated in millimeters. The average of the reading was taken to be the zone of inhibition of the bacterial isolates in question at that particular concentration.

Determination of Anti-Fungal Properties of The Extracts

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Statistical Evaluation

Data analysis and experimental design**:** The phytochemical and antimicrobial testing were statistically analyzed using a 3-factorial or split plot in Completely Randomized Design mean separation was by Duncan's New Multiple Range Test.

RESULTS

The result of the phytochemical analysis reveals the presents of alkaloid, tannin, steroids, flavonoid, terpenes, saponnin and cardiac glycoside. Quantitative and qualitative analysis of*Pterocarpus santalinoides* plant extracts were determined using different solvent of ethanol and methanol. In ethanolic extract, terpenes with (2.64 $\pm$ 0.02) was highest on the stem bark, leaving steroid (1.0 $\pm$ 0.02) as the least. In methanolic extract, alkaloid had (2.6$\pm$ 0.01), while cardiac glycoside was the least (1.90 $\pm$ 0.02) on the same stem bark Table 1.

Table1a. **Quantitative phytochemical analysis of the stem bark extract (%)**

Phytochemicals	Ethanol	Methanol
Alkaloid	2.61 $\pm$ 0.02	2.60 $\pm$ 0.01
Saponnin	2.50 $\pm$ 0.01	2.53 $\pm$ 0.02
Glycoside	2.50 $\pm$ 0.03	1.90 $\pm$ 0.02
Flavonoids	2.00 $\pm$ 0.01	1.83 $\pm$ 0.02
Terpenes	2.64 $\pm$ 0.02	2.10 $\pm$ 0.02
Tannins	1.52 $\pm$ 0.02	1.02 $\pm$ 0.02
Steroid	1.00 $\pm$ 0.02	1.80 $\pm$ 0.02

Table 1b. Qualitative Phytochemical Analysis of *Pterocarpus santalinoides* on stem bark extract

Phytochemical	Ethanol	Methanol
Alkaloid	+	+
Saponnin	+	+
Glycoside	+	+
Flavonoids	+	+
Terpenes	+	+
Tannins	+	+
Steroid	+	+

On the leave extract, terpenes was (2.63 $\pm$ 0.02) as highest in ethanolic extract while tannin had (1.22 $\pm$ 0.01) the least respectively. In methanolic extract, alkaloid was (2.22 $\pm$ 0.02) highest with tannin (1.62 $\pm$ 0.02) the least Table 2. The summary of the microbial identification of the isolates are presented in Table 3,4. All the bacterial isolated were; *E-coli, S.aureus, B. subtilis, K. Pneumonia and P.aeruginosa,* and fungal organisms: *A. niger, A. flavus, T. rubrum, M. gypseum* showed an appreciable inhibiting effect on ethanolic extractas well as methanolic extract of the plant [Table 5].With the ethanolic extract of the leaves, at concentrates 75mg/ml, the most susceptible organism (inhibition diameter >25mm) were ;

Table 2a. Percentage quantificationof phytochemical analysis of Pterocarpus santalinoides on leaf extract

Phytochemicals	Ethanol	Methanol
Alkaloid	2.20 ±0.01	2.22 ±0.02
Saponnin	1.40 ±0.02	1.85 ±0.01
Glycoside	1.48 ±0.02	2.05 ±0.02
Flavonoids	1.75 ±0.01	1.86 ±0.01
Terpenes	2.63 ±0.02	1.76 ±0.01
Tannins	1.22 ±0.01	1.62 ±0.02
Steroid	1.73 ±0.01	1.83 ±0.01

Table 2b. Qualitative Phytochemical Analysis of Pterocarpussantalinoides on leaf extract

Phytochemical	Ethanol	Methanol
Alkaloid	+	+
Saponnin	+	+
Glycoside	+	+
Flavonoids	+	+
Terpenes	+	+
Tannins	+	+
Steroid	+	+

KEYS
(+) = Presence
(-) = Absence

B. subtilis, P.aeruginosa, S.aureus, and E-coli, while the least susceptible isolates (inhibition diameter <25mm) was *K. pneumonia* Table 5. In ethanolic of the stem bark, at concentration of 40mg/ml, *M. gypseum* had (35 ± 0.00) zone of inhibition as highest; while *A. niger* was the least (22±0.00)

With the ethanolic extract, the MIC ranged from 0.1mg/ml on E-coli, *S.aureus, P.aeruginosa, K.Pneumonia* and *B. subtilis* to 100mg/ml. On the stem bark, *S.aureus* and E-coli were highest (3.65 ± 0.00) and (3.12 ± 0.01) on 100mg/ml; while *K. Pneumonia* and *B.subtilis* were least (1.0 ± 0.02) and (1.1 + 0.01) ethanolic extract on 0.1mg/ml concentration respectively.

The leave extract of ethanol was highest (3.8 ± 0.00) against E-coli on 100mg/ml concentration than other concentrations [Table 9]. On the methanolic extract, the leaves had (6.0± 0.01) on 100mg/ml concentration with E-coli, while *K. Pneumonia* and *B. subtilis* had none on 0.1mg/ml concentrate [Table 10].

DISCUSSION

The results of preliminary phytochemical screening of extract and metabolites were presented in Table 1,2, which showed that the extract contained flavonioids, alkaloid, terpenes, tannins, saponnin and cardiac glycosides (Nwokonkwo, 2009; Nwokonkwo, 2013). These results are in good agreement with a similar study earlier conducted by Ajaiyeoba *et al;* (2003).

The presence of the mentioned phytochemicals suggests that the extract and/or its metabolite possess anti microbial potentials against several human pathogens. This is because the different phytochemical compounds have been linked with various bioactive elements Ibekwe (2009).

Table 3. Morphological and Identification of Bacteria Organisms

Nature of Colony formation	Gram Reaction & Microscopy	catalase	Methyl Red	Citrate	Indole	Coagulase	Oxidase	Urease	Sucrose	Maltose	Lactose	Suspected organism
Pink colonies with heavy edge	Gram negative short rods in singles, clusters and scattered	-	+	-	+	-	-	-	AG	AG	AG	*Escherichia coli*
Smooth glittering surface with entire edge	Gram positive cocci in cluster	-	-	+	-	+	-	-	AG	AG	AG	*Staphylococcus aureus*
Cocci in cluster on the slide	Gram negative Cocci in clusters	+	+	+	-	-	-	+	AG	-	AG	*Klebsiella pneumonia*
Cocci in cluster on the slide	Gram negative rod	-	-	+	-	-	-	-	-	+	-	*Pseudomonas aeruginosa*
Smooth suface milky white	Gram positive rods	+	-	+	+	-	-	+	-	A	-	*Bacillus subtilis*

KEYS
+ = Positive reaction: - = Negative reaction ; A = Acid Production; AG = Acid and Gas production

Table 6. At 50mg/ml concentration *P. aeruginosa* was highest (29 ± 0.00), while *S. aureus* was (20 ± 0.01) the least on the methanolic extract Table 7. The zone of inhibition at 20mg/ml was highest (31 ± 0.01) for *M. gypseum,* while *A. niger* was the least (17 ±0.01) Table 8. The result of the minimum inhibitory concentration are presented in Table 9, 10, shows that the values of the different extract ranged between 0.1mg/ml and 100mg/ml.

The ethanol extract exhibited inhibit activities that were found to be little higher than methanolic extract on all the test organism. Although with a slight different. It can be therefore inferred that the active principle of the plant herb may be more soluble in ethanol as employed in ethno medicine Dike (2010). Despite the ethanolic extract exhibited higher inhibitory activities than methanol extract, the antimicrobial activity was low.

Table 4. Morphological and Identification of Fungal Isolates

Microscopic Characteristics	Colour Colony	Reverse Side	Texture	Nature of Growth	Probable Organisms
Septate hyphae unbranched conidiosphores and smooth condiosphores arising from a specialized foot cell	Black powdery suface myceliated and spreading	Yellow	Velvety	Rapid with a filamentous growth	*Aspergillus niger*
Formation of hyphae and arthrosphore, arranged linearly in edothrix	Milky spots surface	Milky	Rough	Rapid with hyphal growth	*Trichophyton rubrum*
Formation of fluorescent, hyphae and arthrosphores	Diffussing red or white pigmentation	Non pigmented	Rough	Slow growth with fluorescents	*Microsporium gypseum*
Septate hyphae unbranched, conidiosphore	Black powdery surface	White	Velvety	Rapid with septate body	*Aspergillus flavus*

Table 5. Zone of inhibition in (mm) of the ethanol extract *P santalinoides* of the leave at various concentrations (mg/ml)

Organisms	5	10	20	25	40	50	75(mg)
E-coli	12±0.01	13±0.01	16±0.01	22±0.01	23±0.01	26±0.01	28.±0.02
S.aureus	14±0.01	16±0.01	20±0.01	28±0.02	28±0.01	28.2±0.01	29.5±0.02
P.aeruginosa	15±0.01	18±0.01	20±0.01	22±0.01	23±0.01	29.0±0.00	30.2±0.02
K.pneumonia	14±0.01	16±0.01	20±0.01	20±0.02	20.5±0.01	21±0.00	22±0.01
B. subtilis	12±0.01	13±0.01	15±0.01	20±0.00	21±0.01	22.2±0.02	30±0.01
A.niger	18.4±0.01	12±0.00	18±0.01	19±0.01	21±0.00	22±0.01	25±0.01
A. Flavus	15±0.02	17±0.00	24±0.01	23±0.01	27±0.02	26±0.01	27±0.01
T. rubrum	28±0.01	29±0.02	29.8±0.00	28±0.01	30±0.01	30±0.01	31±0.01
M. gypseum	32.2±0.00	33±0.01	34±0.02	34±0.01	35±0.00	35±0.01	36±0.01

Table 6. Zone of inhibitions in (mm) of the ethanol extract *P santalinoides* of the Stem Bark at various concentrations (mg/ml)

Organisms	5	10	20	25	40	50	75(mg)ss
E-coli	12±0.01	14±0.01	18±0.01	22±0.01	24±0.01	27±0.01	28.5±0.02
S.aureus	12±0.01	15±0.01	17±0.01	28±0.02	27±0.01	29±0.01	29.8±0.02
P.aeruginosa	12±0.01	16±0.01	18.5±0.01	30±0.01	30±0.01	28.2±0.00	29±0.02
K.pneumonia	13±0.01	16±0.01	20±0.01	21±0.01	22±0.01	24.2±0.00	26±0.01
B. subtilis	12±0.01	14±0.01	22±0.01	29±0.00	29±0.01	28±0.02	30.5±0.01
A.niger	18.6±0.01	25±0.00	20±0.01	20±0.01	22±0.00	23±0.01	24±0.01
A. flavus	20±0.02	27±0.00	25±0.01	23±0.01	26±0.02	27±0.01	28±0.01
T. rubrum	30±0.01	33±0.02	29±0.00	27±0.01	27±0.01	28±0.01	30±0.01
M. gypseum	32.5±0.00	34±0.01	34±0.02	34±0.01	35±0.00	35±0.01	36±0.01

Table 7. Zone of inhibition in (mm) of the Methanol extract of theleave *P santalinoides* at various concentration (mg/ml)

Organisms	5	10	20	25	40	50	75(mg)
E-coli	12±0.01	13±0.01	17±0.01	24.5±0.01	25±0.01	22±0.01	27±0.02
S.aureus	12±0.01	14±0.01	21±0.01	26±0.02	27±0.01	20.02±0.01	29±0.02
P.aeruginosa	12±0.01	16±0.01	20±0.01	28±0.01	28±0.01	29.2±0.00	30±0.02
K.pneumonia	12±0.01	14±0.01	25±0.01	34.5.2±0.02	26±0.01	27±0.00	19±0.01
B. subtilis	13±0.01	16±0.01	23±0.01	25±0.00	23±0.01	28±0.02	19.5±0.01
A.niger	16.5±0.01	22±0.00	18±0.01	19±0.01	20±0.00	23±0.01	25±0.01
A. flavus	18±0.02	25±0.00	23±0.01	23±0.01	24±0.02	24±0.01	25±0.01
T. rubrum	28±0.01	30±0.02	26±0.00	26±0.01	26±0.01	26.5±0.01	27±0.01
M.gypseum	30.5±0.00	32±0.01	33±0.02	35.5±0.01	34±0.00	34±0.01	35±0.01

Table 8. Zone of inhibition in (mm) of the Methanol extract of thestem bark *P santalinoides* at various concentrations (mg/ml)

Organism	5	10	20	25	40	50	75(mg)
E-coli	14±0.01	20±0.01	22±0.01	26±0.01	25±0.01	24±0.01	24±0.02
S.aureus	16±0.01	20±0.01	25±0.01	29±0.02	15±0.01	22±0.01	23±0.02
P.aeruginosa	15±0.01	21±0.01	24±0.01	26.2±0.01	23±0.01	20±0.00	22±0.02
K.pneumonia	15±0.01	20±0.01	24±0.01	26±0.02	24±0.01	18±0.00	19±0.01
B. subtilis	16±0.01	20±0.01	23±0.01	26.5±0.00	24±0.01	21.2±0.02	20±0.01
A.niger	16±0.01	21.8±0.00	17.9±0.01	18±0.01	18±0.00	20±0.01	24±0.01
A. Flavus	17±0.02	24±0.00	22±0.01	22±0.01	23±0.02	24±0.01	26±0.01
T. rubrum	26±0.01	27±0.02	25±0.00	25.5±0.01	25±0.01	26±0.01	28±0.01
M. gypseum	28.5±0.00	32±0.01	31±0.02	32±0.01	33±0.00	34±0.01	35±0.01

Table 9. Minimum Inhibitory Concentration of the ethanol extract of *P. santalinoides (*mm)

	Stem Bark leaves					
Organisms	100mg/ml	10mg/ml	0.1mg/ml	100mg/ml	10mg/ml	0.1mg/ml
E-coli	3.12 ± 0.01	2.2 ± 0.01	2.0 ± 0.00	3.80 ± 0.00	2.6 ±0.00	1.8±0.00
S. aureus	3.65 ± 0.00	2.8±0.00	2.5±0.01	3.70± 0.01	2.0 ±0.00	1.2±0.00
P aeruginosa	2.80±0.00	2.5±0.01	2.3±0.01	2.0±0.00	2.1 ±0.00	1.0±0.00
K.Pneumonia	1.56±0.02	1.20±0.00	1.0±0.00	1.86±0.00	1.0 ±0.00	1.3±0.00
B.subtilis	1.62±0.01	1.26±0.01	1.1±0.01	1.0±0.00	1.0 ±0.00	1.0±0.00

Table 10.Minimum inhibitory concentration of the methanol extract of *P santalinoide*s (mm)

	Stem bark leaves					
Organisms	100mg/ml	10mg/ml	0.1mg/ml	100mg/mI	10mg/ml	0.1mg/mI
E-Coli	3.0±0.00	2.8±0.00	1.5±0.01	6.0±0.01	3.5±0.01	1.5±0.01
S.aureus	3.5±0.01	3.0±0.00	2.5±0.01	4.6±0.01	2.3±0.01	1.0±0.01
*P.aeruginos*a	2.9±0.01	2.5±0.00	2.0± 0.01	3.2± 0.01	2.0±0.01	1.9±0.01
*K.Pneumoni*a	2.6±0.01	2.3+0.00	1.0±0.01	3.6 ±0.01	1.5±0.01	1.2±0.01
*B.subtil*s	5.0±0.01	3.6±0.01	2.2±0.01	1.8±0.01	1.0±0.01	1.2± 0.01

Consequently, the minimum inhibitory concentration (MIC) determined did not show any significant zone of inhibition. The MIC result is traceable to the fact that the two fold serial dilution reduced its initial concentration of 100mg/ml to 10mg/ml and then to 0.1mg/ml which had no visible effect on the organism resulting in no zone of inhibition. A low level of activity at a low extract concentration may suggest that the concentration of the active constituent in the extract are too low for any appreciable antimicrobial activity (Ashebir and Ashenati 2009).

Conclusion

From the result obtained from this work, it can be concluded that the leaf and the stem bark of *Pterocarpu*s *santalinoides* contains tannins, flavonoids, saponnin, glycosides, terpenes, alkaloids and steroids. It has also been confirmed that the stem bark possesses a broad spectrum antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa, Escherichia coli,Staphylococcus aureus, Klebsiella pneumonia* and *Aspergilius species, Trichophyton rubrum and microsporium gypseum* with varying degree of MIC values and inhibitions. The broad spectrum antibacterial activity exhibited by *Pterocarpus santalinoides* in this work may be attributed to the various active component present in it which either due to their individual activity.

The presence of phytochemicals with various pharmacological and biological properties in the extracts determines the medicinal value of the plants as a useful source of drug in ethnomedicine. Moreso, this work lends credence to the fact that these extracts have good phytochemical and antimicrobial qualities, which justify the use in traditional medicine.

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REFERENCES

Adetuyi, A.O. and Akpambang, O.E. 2005. The nutritional Value of sorghum bicolorl stem flour used for infusion drink in nigeria. *Park. J. Sci. Ind. Res*., 49:276-278.

Ajaiyeoba, E.O. and Onocha, P. 2003. Antimicrobial and cyto-toxicity Evaluation of *Buchholziacoriacea* stem bark. Fitoterapia, 74(7-8):706- 709.

Akerele, B.C. and Azoro C. 2002. Antibacterial activity of crude extract of *Azadirachita Indicia* on *Salmonella typhi*.*World Journal of Biotechnology,* 3(1):347-351.

Akinpelu, D.A and Onakoya, T.M. 2006. Antimicrobial activities of medicinal plants used in forklore remedies in south-western Africa. *Afri.J.Trad,CAM;*3:112-115.

Akinyeye, R.O. 2010. Proximate, mineral. Anti-nutrients, phytochemical sreening and amino acid compositions of the leaves of *Pterocarpus mildbraedi harms*. *Electronic Journal of Environmental Agricultural Chemistry*,9 (1-2):1322-1333.

Alexis, V. and Michele M. 2000. Anti- plasmodial activity of plant extract used in West African Medicine. *J. of ethno Pharmacology,* 73(1-2):141-151.

Ama, C.A. 2010. Traditional use of *Pterocarpus Santalinoides* parts, Chief Dr.Ama C. Ama, Trado medical centre, Amajieke, Owerri, Imo State. Nigeria.

Anowi, C.F. and Onyekaba, T.C. 2012. Antimicrobial properties of the methanolic extract of the leaves of Nauclea *latifolia*. *Int.J. Drug Res. Technol*., 2:45-55.

Antia, B.S. and Umoren, 2006. Nutritional and Anti-nutritional Evaluation of Sweet potatoe (*Ipomea batatas*) leaves. *Park.J.nutri*., 2:166-168.

AOAC 2010**.** Official Method of Analysis. Association of Official Analytical Chemistry.15th Ed., Washington D.C, USA, 1:600-792.

Aregheore, E.M. 2012. Nutritive value and inherent Anti-nutritive factors in four indigenous Edible leafy vegetables in Human Nutrition in Nigeria; *A Review. J. Food Resource. Sci.,* 1:1-14.

Coe, F.L. and Worcester 2005. Kidney stone diseases. *J. Clin. Investigation*, 115(10):2598-2608.

constituents of the seeds of *Cussonia bateri* (Jansa Seeds). *Am. J. sci Ind. Res*. 4(4):424-419.

Dike, M.C. 2010. Proximate, phytochemical and Nutrient compositions of some fruits, seed and leaves of some plant species at Umudike, Nigeria. *Journal of Agricultural and Biological Sciences*, 7(6), 7-16.

Ejele, A.E. and Nwokonkwo.D.C.2013. Effects of spoilage on Antimicrobial potential and potential and phytochemical composition of Ipomea Root Extract. *Int. Res. J. of micro*, 4(4):106-112.

Ekeke, B.C. and Okwu, D.E. 2009. Phytochemical Screening and the medical composition of chewing stick in south Eastern Nigeria. *Global Journal of pure and Applied Sciences*, 9(1), 235- 238.

Igoli, J.O. 2005. Traditional medicine practice amongst the igede people of Nigeria, part 11, *International Journal of Biotechnology and Allied Sciences* 4(4):408-412

Iroha, I.R. and Nwuzo, A.C. 2008. Anti microbial activity of extract of *Garcinia kola* against resistant extended beta lactamase producing *E-coli* and *Klebsiella pneumonia*, Nig. *Journal of Microbiology,* 22, 1693-1697.

McGee, H. 2004. On food and cooking: The Science and love of the kitchen. Scribner, New York, pp:17.

Nwokonkwo, D.C. 2013. Physicochemical and phytochemical studies of the

Ogbe, A.O. and Affiku, J.P. 2006. Proximate study, mineral and Anti-nutrient Composition of *Moring oleifera* leaves harvested from Lafia,Nigeria Potential Benefit in poultry nutrition and Health. *J. microbial Biotechnol. food sci.*1:296-308.

Okwu, D.E. and Nnamdi, F.U. 2008. Evaluation of the chemical composition of Dacryoides edulis and Raphia hookeri Mann exudates used in herbal medicine in South Eastern Nigeria. *Afri. J. Trad. Complex. Altima. Med*, 5:194-200.

Oluwole, F.S. and Ogundipe, O.O. 2012. Anti-Inflammatory effect of some common Nigeria Vegetables. *Nig. J. Physiol. Sci*., 18:35-38.

Onwuka, G.I. 2005**.** Food Analysis: Theory and Practice. Naphtali prints, Lagos, Nigeria. PP. 133-134, 144-199.

Osuagwu, G.E. and Emenike, J.O. 2007. Phytochemical and mineral content of the leaves of four Nigeria Pterocarpus species. *International Journal of molecular medicine and advance sciences,* 3(1), 6-11.

World Health Organisation (WHO) 2013. Resolution Promotion and Development of training and research in traditional medicine WHO Document No. 30: 47-49.

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