Original Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITIES OF FRACTIONS OF PLANT PARTS OF *Pterocarpus santalinoides*

ABSTRACT

Aims: The study aims to investigate the antimicrobial activities of the leaves, seeds, bark, and root of *Pterocarpus santalinoides* plant.

Study Design: Agar well diffusion and Agar well dilution methods were used to test the preliminary antimicrobial and minimum inhibitory/bactericidal/fungicidal concentrations respectively of *Pterocarpus santalinoides* plants.

Place and Duration of Study: Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus, Nigeria, between February – October, 2017.

Methodology: Primary extraction and fractionation ~~was~~ were done on the plant parts with methanol, butanol, ethyl acetate, and n-hexane. Agar diffusion method for the primary antimicrobial screening on Muller-Hinton agar (Bacteria) and Sabouraud dextrose agar (Fungi) were used to test the antimicrobial activities of the sixteen (16) samples on some microorganisms; *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Aspergillus niger*, *Microsporon canis*, and *Trichophyton rubrum*. The minimum ~~Inhibitory~~ inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration (MBC/MFC) and percentage inhibition diameter growth (PIDG) of the samples that gave activity were also evaluated.

Results: Twelve (12) samples showed inhibitory activity on at least one or more of the test organisms. The MIC range observed for the extracts and fractions that gave activity was 12.5 – 100 mg/ml. The n-hexane fraction of the plant root gave the best value of 12.5 mg/ml against ~~*Microsporon*~~ *M. canis*. The best MBC/MFC value of 25 mg/ml was observed with the ethyl acetate fraction of the bark (against *E. coli* and *M. canis*) and the n-hexane fraction of the root (against *M.* canis). The result showed *S. typhi* to be the most sensitive organism to the metabolites of *P. santalinoides*. ~~Extended~~ Extended-spectrum activity was observed with the ethyl acetate fraction of the bark against three (3) of the test organisms; *S. typhi*, *E. coli*, and *M. canis*. The determination of PIDG values for the test organisms against the plants' extracts/fractions showed that crude methanol extract (28.57%) and ethyl-acetate fraction (0.14%) of the leaves, ~~Butanol~~ butanol fraction (0.14%) of the root (all against *Salmonella typhi*) were the most potent test samples.

Conclusion: The result indicates that the plants may have potential medicinal values and suggests its use in traditional medicine.

Keywords: Pterocarpus santalinoides; Antimicrobial; Methanol; n-hexane; Ethyl acetate; Butanol.

1. INTRODUCTION

Medicinal plants have been of great value to mankind. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20 – 25 years [1]. Higher plants have been used for centuries as remedies for human diseases [2, 3]. Different plant parts have also been used for various forms of diseases and infections. This has encouraged research into the screening of plants for antibacterial and antifungal activities [4].

The increased material worth of medical treatment and their strong physiological or chemical effects, contribute to the reason why individuals make use of herbal therapy. The increasing demand of plant extracts used in the cosmetic, food and pharmaceutical industries suggests that systematic studies of medicinal plants are very important in order to find active compounds and their use as a medicine for curing various diseases.

Pterocarpus santalinoides is a tree 9 - 12 m tall, 1 m diameter at breast height (DBH), with low straggling branches. Bark thin and flaking in small patches, slash yellowish-white exuding drops of red gum [5]. It is a shade tolerant tree commonly found along the riverine forests in Africa and tropical South America [6].

In Nigeria, many indigenous plants including ~~*Pterocarpus*~~ *P. Santalinoides are* used as food or medicine. The tender leaves are used as vegetable in soup preparing, while the stem bark is used in making pepper soup. ~~*Pterocarpus*~~ *P. santalinoides* is a plant believed to possess potent antibacterial properties in ethno medicine. The plants are used in treating rheumatism, diarrhea, dysentery, cough, asthma, diabetes, malaria, elephantiasis, cold, and others [7]. The use of the plant leaves in treating skin disease such as eczema, candidiasis, and acne have been reported [8]. The use of the concoction made from its root in treating asthmatic patients ~~have~~ has also been reported [9]. It is also used in treating diarrhea which is a major cause of death as it has a proven anti enteropoling activity in traditional medicine [10].

This research is aimed at investigating the antimicrobial activities of various parts of the plant against some indicative organisms implicated in their traditional use.

2. MATERIALS AND METHODS

2.1 Plants Materials

The fresh leaves, seeds, bark and roots of ~~*Pterocarpus*~~ *P. santalinoides* were sourced in September 2016 from Okofia - Otolo Nnewi, Anambra ~~state~~State, Nigeria, latitude $5^0$58'48.86'' and longitude $6^0$54'30.78''. They were identified and authenticated by a Curator in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria.

2.2 Culture Media and Reagents

Culture media used were Nutrient Broth, Mueller-Hinton agar (Oxoid Limited, England) and Sabouraud dextrose agar (Titan Biotech, India). Culture media were prepared according to the instructions of the manufacturers.

Reagents used include McFarland 0.5 turbidity standard (prepared from ~~Barium~~ barium chloride, ~~Sulfuric~~ sulfuric acid and water), sodium hypochlorite solution, and dimethyl sulphoxide (DMSO) (Triveni Chemicals, India).

2.3 Equipment

Equitron partially automatic autoclave (Medica Instrument Manufacturing CO., India), hot air oven (Genlab, UK), incubator (Genlab, UK), electronic weighing balance (Ohaus Corp., USA).

2.4 Microbial Test Organisms

The microorganisms used in this study were two bacteria isolates (*Escherichia coli* and *Salmonella typhi*) stored at 4 – 8 ºC in Mueller-Hinton Agar slants and four fungi isolates (*Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum*, and *Microsporon canis*). These were clinical isolates previously purified and standardized to McFarland. Their susceptibility to commonly used antibiotics was already established. The isolates were obtained from the Department of Pharmaceutical Microbiology & Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria.

2.5 Extraction of Plant Materials

The different plant parts of ~~*Pterocarpus*~~ *P. santalinoides* were prepared using the method of (mention the author surname followed by et al.) [11]. They were dried at room temperature (25 ^{o}C), grounded into a

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fine powder using laboratory grinding mill and stored in a cool and dry place. Total extraction of each plant materials ~~were~~ was prepared by mixing with solvent (methanol) in the ratio of 1:10 (plant material/solvents) in a cold maceration system. The plant materials were soaked in 1000 ml solvent in conical flasks for 12 h~~ou~~rs. The extracts were then filtered using Whatman No. 1 filter paper and solvents removed through evaporation under reduced pressure at 45 ^{o}C using a rotary evaporator (Yamato, USA). The extracts were kept in stoppered sample vials at 4 ^{o}C until they were used.

2.6 Fractionation of Crude Methanol Extracts

The crude methanol extracts of the different parts of the plant were fractionated using the solvent-solvent method with n-hexane, ethyl acetate, and butanol in order of increasing polarity.

2.7 Percentage Yield Determination

The percentage yield (%, w/w) from all the dried crude methanol extracts was calculated using the formula outlined below:

$$\text{Percentage (\%) yield} = \frac{\text{Weight of extract}}{\text{Weight of dried plant material}} \times 100 \quad \text{.....Equation 1}$$

2.8 Preliminary Antimicrobial Assay

The ~~Preliminary~~ preliminary antimicrobial assay for each of the crude extracts and their respective fractions was carried out using the agar well diffusion assay as described by (mention the author surname followed by et al.) [12]. The antimicrobial activity of the plant parts (i.e. crude extracts and fractions) were tested against standard clinical isolates (two bacteria and four fungi isolates) as listed above. A 0.5 McFarland standard bacterial/fungal suspension of each of the test isolates were prepared and these formed both the bacterial and fungal stock solutions used in the agar well diffusion assays as outlined below.

2.9 Agar Well Diffusion Assay

Briefly, the media, Mueller-Hinton agar, MHA (Oxoid, USA) and Sabouraud dextrose Agar, SDA, were prepared and treated according to the manufacturer's specification. MHA (38 g) and SDA (65 g) respectively were mixed with 1L of sterile distilled water and sterilized at 121 ^{O}C for 15 mins. The media was allowed to cool to 50 ^{O}C and later transferred into 90 mm sterile agar plates and left to set. The sterile MHA and SDA plates were inoculated with the test culture from each of the test suspensions, thereafter, 20 ml of the sterile molten agar cooled to 50 ^{O}C was added then to the plate and was rocked clockwise and anti-clockwise to ensure even distribution of the test organism. This was done to obtain uniformity of the inoculums. A sterile cork borer was used to make wells (6 mm in diameter) on each of the MHA and SDA plates, respectively. Aliquots of 60 µl of the stock concentration (100 mg/ml) of each extracts and fractions, reconstituted in ~~dimethyl sulphoxide (~~DMSO~~)~~ were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin (5 µg) and miconazole (50 µg/ml) served as the positive controls for the bacteria and fungi respectively, while DMSO served as the negative control. The cultures were incubated at 37 ^{O}C for 18 - 24 h~~ou~~rs for the bacterial plates and 25 – 27 ^{O}C for 48 h~~ou~~rs for the fungal plates, respectively. The antimicrobial potential for each extract and fraction was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each extract and fraction, three replicates were conducted against each organism. Each extract and fraction was tested against all the bacterial and fungal isolates.

Furthermore, based on the recorded activity, the extracts and fractions that showed activity were subjected to minimum inhibitory concentration (MIC) and maximum biocidal concentration (MBC) ~~Determinations~~determinations.

2.10 Anti-dermatophyte Activity

The anti-dermatophyte activity was conducted using the method as described by (mention the author surname followed by et al.) [13]. A sterile swab was used to aseptically inoculate each of the fungal suspension (~~*Trichophyton* *T. rubrum and* *microsporon* *M. canis*~~) on the surface of sterilized Sabouraud dextrose agar. The tests were carried out using a stock concentration of 100 mg/ml prepared by dissolving 200 mg of the crude extracts and fractions into 2 ml of DMSO. A well of 6 mm diameter was made in the agar plate then loaded with 60 µl of each of the stock concentration of the crude extracts and fractions of the test samples and incubated at 28 ± 2 °C for 15 - 20 days. The inhibition zone was observed and then recorded in millimeters using a transparent ~~metro~~meter rule. The test was conducted in triplicate and results presented as mean. Miconazole 50 µg/ml served as the standard positive control against the *dermatophytic species.*

2.11 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was interpreted as the lowest concentration of the test samples (extracts and fractions) that inhibited visible growth. ~~-~~The MICs of the active samples were determined by agar dilution method as described by (mention the author surname followed by et al.) [13, 14] for the antimicrobial and anti-dermatophytic activities, respectively. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test crude extracts and fractions. In this test, a stock solution of the crude extracts and fractions (2,000 mg/ml) was made, then 2-fold serial dilution was done to get graded dilutions (1000, 500, 250, 125, and 62.5 mg/ml) of each of the crude extract and fraction. Then 1 ml of each of ~~these~~ this concentration was transferred into a sterile petri dish and properly mixed with 9 ml of molten Mueller-Hinton agar and Sabouraud dextrose agar, then cooled to 45 - 50 ^{o}C. After the mixing was done the final concentrations becomes 100, 50, 25, 12.5, and 6.25 mg/ml respectively. Finally, the different test organisms were streaked on the solidified agar properly labeled and incubated at 37 °C for bacteria and 28 ± 2 °C for fungi. The bacteria w~~h~~ere incubated for 18 – 24 hours, while ~~*Candida* *C. albicans*~~ and ~~*Aspergillus* *A. niger*~~ were incubated for 48 hours and the dermatophytes incubated for 15 - 20 days. Each experiment was performed in triplicate.

2.12 Determination of the Minimal Bactericidal/Fungicidal Concentration of the Crude Extracts and Fractions (MBC)

This is the minimal concentration which kills off the cells in a microbial population. The MBC/MFC is the lowest concentration producing no evidence of growth [14]. It is an extension of the MIC procedure (stated above) carried out. Here, the plates that show no visible growth in the MIC test were selected. Then, they are incubated for 48 more hours. Thereafter, the plates were examined for signs of microbial growth.

2.13 Determination of the Percentage Inhibition Diameter Growth (PIDG)

Following the observation for the antimicrobial evaluation, the percentage inhibition diameter growth (PIDG) values were determined according to the equation as below.

$$\text{PIDG (\%)} = \frac{\text{Diameter of sample} - \text{Diameter of control}}{\text{Diameter of control}} \times 100 \quad \text{.....equation 2}$$

2.14 Data Analysis

The results were expressed as mean. Statistical analysis was carried out using ~~one~~ one-way analysis of variance (ANOVA) and SPSS (version 20) statistical program. The obtained results were considered significant at P = 0.05.

3. RESULTS

3.1 Extraction, Fractionation, and Percentage Yield

Each of the leaves, seeds, barks, and roots of ~~*Pterocarpus*~~ *P. santalinoides* were extracted with methanol as the primary solvent. This yielded four (4) crude methanol extracts of the plant samples. The leaves (8.27 %) gave the highest percentage yield as depicted in (Table 1). This was followed by the bark (5.97 %). The least value was observed with the seeds (1.97 %).

The solvent-solvent fractionation of the four (4) crude methanol extract of the plant, ~~*Pterocarpus*~~ *P. santalinoides* with n-hexane, ethyl acetate and butanol in their order of increasing polarity gave a total of four (4) fractions each of the three (3) secondary solvents giving a total sample of sixteen ((4) each of the crude methanol extracts, n-hexane, ethyl acetate, and butanol fractions respectively) samples.

Table 1: The percentage yield of crude extracts obtained from *Pterocarpus santalinoides*

Plant Parts	Powdered Plant materials (Wt. in g)	Extract weight (g)	Percentage yield (%)
Leaves	546.10	45.15	8.27
Seeds	500.00	9.87	1.97
Barks	450.00	26.87	5.97
Roots	307.89	15.16	4.92

3.2 Antimicrobial Activity of *Pterocarpus sanatalinoides* Extracts and Fractions against the Test Isolates

The antimicrobial activities of the plant extracts and fractions were tested against six (6) pathogenic cultures comprising of two (2) bacteria strains - *Escherichia coli* and *Salmonella typhi,* and four (4) fungi species - ~~*Candida*~~ *C. albicans*, ~~*Aspergillus*~~ *A. niger*, ~~*Microsporon*~~ *M. canis and* ~~*Trichophyton*~~ *T. rubrum*. The IZDs ~~were~~ was variable ranging from 2 - 9 mm. ~~Broad~~ Broad-spectrum antimicrobial agents (ciprofloxacin and miconazole) were used as ~~a~~ the positive control while ~~Dimethylsulfuroxide~~ dimethylsulfuroxide (DMSO) was used as the negative control.

A total of 16 crude methanol extracts and fractions of the plant parts were tested for their antimicrobial activity among which 12 samples showed inhibitory activity on at least one or more of the test pathogenic microorganisms. In contrast, 4 samples (PSL n-hexane, PSB Crude, PSB ~~Butanol~~butanol, and PSB n-hexane) showed no activity against any of the selected microorganism at the tested concentration. The result showed that ethyl acetate fraction of the plant bark (PSB Ethyl acetate) has antimicrobial activity against 3 of the tested organisms (~~*Salmonella*~~ *S. typhi*, *E. coli*, and ~~*Microsporon*~~ *M. canis*).

The ~~Ciprofloxacin~~ ciprofloxacin (5 µg/ml) conventional drug used as a control was active against the bacterial isolates with mean ~~inhibition zone diameter (~~IZD~~)~~ of 7 mm against *S. typhi* and 5 mm against *E. coli*. The Miconazole (50 µg/ml) used as a control for fungal isolates was active against all the fungal isolates with mean ~~inhibition zone diameter (~~IZD~~)~~ of 18 mm for ~~*Candida*~~ *C. albicans* and ~~*Microsporon*~~ *M.canis* and 9 mm for ~~*Aspergillus*~~ *A. niger* and ~~*Trichophyton*~~ *T. rubrum*, respectively. This reveals that the tests organisms used in the course of this work were susceptible strains. The DMSO serving as the negative control had no inhibition activity on any of the test organisms. The results of the antimicrobial activity of the plant parts are summarized in Tables 2.

Table 2: Mean Inhibition Zone Diameter (IZD) mm of the preliminary antimicrobial activity of the crude extracts and fractions of *Pterocarpus santalinoides* against some selected clinical isolates.

Samples (100 mg/ml)	ST	EC	CA	AN	MC	TR

Leaves	**PSL Crude**	9	0	0	0	0	0
	PSL Butanol	3	0	0	0	0	0
	PSL Ethyl acetate	7	0	0	0	0	0
	PSL n-hexane	0	0	0	0	0	0
Seeds	**PSS Crude**	0	5	0	0	0	0
	PSS Butanol	2	0	0	0	0	0
	PSS Ethyl acetate	5	0	0	0	0	0
	PSS n-hexane	4	0	0	0	0	0
Barks	**PSB Crude**	0	0	0	0	0	0
	PSB Butanol	0	0	0	0	0	0
	PSB Ethyl acetate	4	3	0	0	4	0
	PSB n-hexane	0	0	0	0	0	0
Roots	**PSR Crude**	0	2	0	0	0	0
	PSR Butanol	7	0	0	0	0	0
	PSR Ethyl acetate	6	0	0	0	0	0
	PSR n-hexane	0	0	0	2	6	0
Control	**Ciprofloxacine 5 µg/ml**	7	5	nd	nd	nd	nd
	Miconazole 50 µg/ml	Nd	Nd	18	9	18	9
	DMSO	0	0	0	0	0	0

Key: nd: not determined; ST: *Salmonella typhi;* EC: *Escherichia coli*; CA: *Candida albicans*; AN: *Aspergillus niger*, MC: *Microsporon canis*, TR: *Trichophyton rubrum*, PSL Crude: *Pterocarpus santalinodes* Leaf (Crude extract); PSL Butanol: *Pterocarpus santalinodes* Leaf (Butanol fraction); PSL Ethyl acetate: *Pterocarpus santalinodes* Leaf (Ethyl acetate fraction); PSLn-hexane: *Pterocarpus santalinodes* Leaf (n-hexane fraction), PSS Crude: *Pterocarpus santalinodes* Seed (Crude extract); PSS Butanol: *Pterocarpus santalinodes* Seed (Butanol fraction); PSS Ethyl acetate: *Pterocarpus santalinodes* Seed (Ethyl acetate fraction); PSS n-hexane: *Pterocarpus santalinodes* Seed (n-hexane fraction), PSB Crude: *Pterocarpus santalinodes* Bark (Crude extract); PSB Butanol: *Pterocarpus santalinodes* Bark (Butanol fraction); PSB Ethyl acetate: *Pterocarpus santalinodes* Bark (Ethyl acetate fraction); PSB n-hexane: *Pterocarpus santalinodes* Bark (n-hexane fraction), PSR Crude: *Pterocarpus santalinodes* Root (Crude extract); PSR Butanol: *Pterocarpus santalinodes* Root (Butanol fraction); PSR Ethyl acetate: *Pterocarpus santalinodes* Root (Ethyl acetate fraction); PSR n-hexane: *Pterocarpus santalinodes* Root (n-hexane fraction); Standard error: ST: 0.7.

The comparison of the activity of the extracts and fractions of ~~*Pterocarpus*~~ *P. santalinoides* according to the parts showed that the extracts and fractions significantly (p=0.05) inhibited some of the test organisms. For the leaves; the crude methanol extract (PSL Crude) had a better antimicrobial activity to the only sensitive organism which is ~~*Salmonella*~~ *S. typhi* followed by ethyl acetate fraction (PSL ~~Ethyl~~ ethyl acetate) and ~~Butanol~~ butanol fraction (PSL ~~Butanol~~butanol) respectively. The n-hexane fraction (PSL n-hexane) had no inhibition activity. This result indicates that the active agents in the plant leaves against this organism are chiefly polar compounds. For the Seeds; the ethyl acetate fraction (PSS ~~Ethyl~~

ethyl acetate) had the best inhibition activity against *S. typhi* followed by n-hexane (PSS n-hexane) and butanol (PSS ~~Butanol~~butanol) fractions respectively. The crude methanol extract (PSS Crude) was not active against *S. typhi*, however, was the only sample of the plant seeds active against *E. coli*. No part of the plant seeds has inhibition activity against *C. albicans*, *A. ~~nig~~ niger*, *M. canis*, and *T. rubrum*. From this result, the phytochemicals in the seeds active against *S. typhi* are mainly non-polar compounds, however, the compounds active against *E. coli* are polar compounds. It also showed that after fractionation of the crude methanol extract (PSS Crude), the active principles in the seed against *E. coli* was lost, which indicates an antagonistic effect of the fractionation process on the active principles. No part of the bark had inhibition activity on any of the tested organisms except for ethyl acetate fraction (PSB ethyl acetate) which had activity against *S. typhi*, *E. coli*, and *M. canis*. This result showed that the active principles in the plant are bipolar compounds. This is logically in accordance to some work done [15] that tested the antimicrobial activities of the ethanol and water extracts of *P. santalinoides* bark against organisms including *S. typhi* and *E. coli*, of which the water extracts (a polar solvent) did not inhibit these test isolates at the concentration used. The ethanol extracts only marginally inhibited the organisms. For the roots, the crude methanol extract (PSR Crude) and the n-hexane fraction (PSR n-hexane) had no activity against *S. typhi*. The butanol fraction (PSR Butanol) had the best activity against *S. typhi*. Only the crude methanol extract (PSR Crude) showed inhibition activity against *E. coli*. The best antimicrobial activity was observed with the n-hexane fraction (PSR n-hexane).

Comparison of the susceptibility of all the test organisms to the various extracts/fractions of the plant parts, indicated that ~~*Salmonella*~~ *S. typhi* appeared to be the most sensitive organism to metabolites from ~~*Pterocarpus*~~ *P. santalinoides* with 9 of the extracts/fractions showing inhibitory activity and the best activity was observed with the crude methanol extract of the plant leaves (PSL Crude) were IZD of 8 mm, MIC of 50 mg/ml and MBC of 100 mg/ml were recorded. In contrast, ~~*Candida*~~ *C. albicans* and ~~*Trichophyton*~~ *T. rubrum* were found to be the most resistant organisms where none of the extracts/fraction showed antimicrobial effect at tested concentrations.

Three samples (PSS Crude, PSB ~~Ethyl~~ ethyl acetate, and PSR Crude) showed antimicrobial activities against ~~*Escherichia*~~ *E. coli* with the best activity observed for crude methanol extract of the plant seed (PSS Crude) where IZD of 5 mm and MIC of 100 mg/ml were recorded. No part of the plants' leaves extract/fractions had activity against *E. coli*. This is similar to work done by [16], who evaluated the antimicrobial activities of the methanol extracts of the plant leaves against *S. aure~~a~~us* and *E coli*, of which *E, coli* showed no activity at all concentrations (up to a maximum concentration of 50 mg/ml). ~~*Microsporon*~~ *M. canis* was inhibited by only two fractions (PSB ~~Ethyl~~ ethyl acetate and PSR n-hexane), while ~~*Aspergillus*~~ *A. niger* was inhibited by only one fraction (PSR n-hexane).

The ethyl acetate fraction of the plant bark (PSB ~~Ethyl~~ ethyl acetate) showed activities against 3 of the tested isolates producing IZD of 4 mm against ~~*Salmonella*~~ *S. typhi*, 3 mm against ~~*Escherichia*~~ *E. coli* and 4 mm against ~~*Microsporon*~~ *M. canis* showing an extended activity than the other extracts/fractions with activities against 2 ~~Gram~~ Gram-negative and 1 fungi isolates. This is similar to a work done by [17], ~~were~~ was a broad spectrum activity of the plant bark was reported as was observed in this study. However, the best antifungal activity was observed for n-hexane fraction of the plant root (PSR n-hexane) where an IZD of 2 mm against ~~*Aspergillus*~~ *A. niger* and 6 mm against ~~*Microsporon*~~ *M. canis* were recorded.

The result of this study showed that the various extracts and fractions of the plant have antimicrobial activity against ~~*Salmonella*~~ *S. typhi*. Similarly, [18] reported the activity of this plant to this organism.

3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of *Pterocarpus santalinoides* Extracts and Fractions

The lowest concentration of the extracts and fractions of the plant parts at which no growth of microorganism was observed upon visual observation after incubation was considered the MIC value. The range of MIC of the plant~~s~~ extracts and fractions recorded was 12.5 – 100 mg/ml. The n-hexane

fraction of the root gave the lowest MIC value (12.5 mg/ml) against ~~*Microsporon* *M.*~~ *canis*, followed by ethyl acetate fraction of the bark with 25 mg/ml against *E. coli* and ~~*Microsporon* *M.*~~ *canis*.

The lowest concentration of the extracts and fractions of the plant parts at which no growth of microorganism was observed upon visual observation after further incubation of the MIC plates were recorded as the Minimal Bactericidal Concentration (MBC) for bacteria isolates and Minimum Fungicidal Concentration (MFC) for fungal isolates. This is shown in Table 4. The ethyl acetate fraction of the bark (PSBE) and the n-hexane fraction of the plant root (PSRn) gave the lowest value (25 mg/ml) against *E. coli* and ~~*Microsporon* *M.*~~ *canis*, respectively.

3.4 Determination of the Percentage Inhibition of Diameter Growth (PIDG)

The determination of the PIDG for all the test organisms showed the most potent samples using the samples with higher ~~Inhibition Zone Diameter (~~IZD~~)~~ (Table 5) compared to the positive control used (5 µg/ml Ciprofloxacin) as threshold/standard.

Table 3: Minimum Inhibitory Concentration (mg/ml) of the crude extracts and fractions of *Pterocarpus santalinoides* against tests isolates

Isolate	PSLC	PSLB	PSLE	PSLn	PSSC	PSSB	PSSE	PSSn	PSBC	PSBB	PSBE	PSBn	PSRC	PSRB	PSRE	PSRn
ST	50	100	100	-	-	-	50	100	-	-	100	-	-	100	50	-
EC	50	-	-	-	100	-	-	-	-	-	25	-	-	-	-	-
CA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100
TR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MC	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-	12.5

Key: ST: *Salmonella typhi;* EC: *Escherichia coli*; CA: *Candida albicans*; AN: *Aspergillus niger*, MC: *Microsporon canis*,TR: *Trichophyton rubrum*, PSLC: *Pterocarpus santalinodes* Leaf (Crude extract); PSLB: *Pterocarpus santalinodes* Leaf (Butanol fraction); PSLE: *Pterocarpus santalinodes* Leaf (Ethyl acetate fraction); PSLn: *Pterocarpus santalinodes* Leaf (n-hexane fraction), PSSC: *Pterocarpus santalinodes* Seed (Crude extract); PSSB: *Pterocarpus santalinodes* Seed (Butanol fraction); PSSE: *Pterocarpus santalinodes* Seed (Ethyl acetate fraction); PSSn-hexane: *Pterocarpus santalinodes* Seed (n-hexane fraction), PSBCrude: *Pterocarpus santalinodes* Bark (Crude extract); PSBB: *Pterocarpus santalinodes* Bark (Butanol fraction); PSBE: *Pterocarpus santalinodes* Bark (Ethyl acetate fraction); PSBn: *Pterocarpus santalinodes* Bark (n-hexane fraction), PSRC: *Pterocarpus santalinodes* Root (Crude extract); PSRB: *Pterocarpus santalinodes* Root (Butanol fraction); PSRE: *Pterocarpus santalinodes* Root (Ethyl acetate fraction); PSRn: *Pterocarpus santalinodes* Root (n-hexane fraction).

Table 4: Minimum Bactericidal/Fungicidal Concentration (mg/ml) of the crude extracts and fractions of *Pterocarpus santalinoides* against tests isolates

Isolate	PSLC	PSLB	PSLE	PSLn	PSSC	PSSB	PSSE	PSSn	PSBC	PSBB	PSBE	PSBn	PSRC	PSRB	PSRE	PSRn
ST	100	100	100	-	-	-	50	100	-	-	-	-	-	-	100	-
EC	100	-	-	-	-	-	-	-	-	-	25	-	-	-	-	-
CA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MC	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-	25

Key: ST: *Salmonella typhi;* EC: *Escherichia coli*; CA: *Candida albicans*; AN: *Aspergillus niger*, MC: *Microsporon canis*,TR: *Trichophyton rubrum*, PSLC: *Pterocarpus santalinodes* Leaf (Crude extract); PSLB: *Pterocarpus santalinodes* Leaf (Butanol fraction); PSLE: *Pterocarpus santalinodes* Leaf (Ethyl acetate fraction); PSLn: *Pterocarpus santalinodes* Leaf (n-hexane fraction), PSSC: *Pterocarpus santalinodes* Seed (Crude extract); PSSB: *Pterocarpus santalinodes* Seed (Butanol fraction); PSSE: *Pterocarpus santalinodes* Seed (Ethyl acetate fraction); PSSn-hexane: *Pterocarpus santalinodes* Seed (n-hexane fraction), PSBCrude: *Pterocarpus santalinodes* Bark (Crude extract); PSBB: *Pterocarpus santalinodes* Bark (Butanol fraction); PSBE: *Pterocarpus santalinodes* Bark (Ethyl acetate fraction); PSBn: *Pterocarpus santalinodes* Bark (n-hexane fraction), PSRC: *Pterocarpus santalinodes* Root (Crude extract); PSRB: *Pterocarpus santalinodes* Root (Butanol fraction); PSRE: *Pterocarpus santalinodes* Root (Ethyl acetate fraction); PSRn: *Pterocarpus santalinodes* Root (n-hexane fraction).

The best MIC value was observed with n-hexane fraction of the plant root (PSRn) against ~~*Microsporon* *M.*~~ *canis*, followed by ethyl acetate fraction of the plant bark (PKBE) with MIC of 25 mg/ml against *E. coli and* ~~*Microsporon* *M.*~~ *canis*. The best MBC value was observed with ethyl acetate fraction of the bark (PSBE) and n-hexane fraction of the roots (PSRn) were 25 mg/ml was observed against *E. coli* and ~~*Microsporon* *M.*~~ *canis*, respectively.

The Research conducted by [6], on the preliminary phytochemical analysis of methanolic extract of ~~*Pterocarpus* *P.*~~ *santalinoides* leaves showed the presence of alkaloids, anthocyanins, carotenoids, flavonoids, resins, saponins, steroids, terpenoids and tannins, while [19] revealed the presence of leucoanthocyanins, coumarins, flavonoids, mucilage, saponins, and tannins. The presence of alkaloids, flavonoids, tannins, saponins, phenolics, and cyanogenic glycosides were revealed by [15] in the ~~ethanoloc~~ ethanolic extracts of the plant stem bark. No study was seen on the phytoconstituents of the plant's seed and root, but relatively will have the compounds as listed above, though environmental factors affect~~s~~ plants phytoconstituents. The antibacterial activities observed with these plant extracts and samples can be ~~attribiuted~~attributed to these secondary metabolites [15, 20]

Table 5: PIDGs of test organisms towards different extracts and fractions of *Pterocarpus santalinoides*

Samples (100 mg/ml)		ST (%)	EC (%)	CA (%)	AN (%)	MC (%)	TR (%)
Leaves	**PSL Crude**	28.57	*	*	*	*	*
	PSL Butanol	-57.14	*	*	*	*	*
	PSL Ethyl acetate	0.14	*	*	*	*	*
	PSL n-hexane	*	*	*	*	*	*
Seeds	**PSS Crude**	*	0	*	*	*	*
	PSS Butanol	-71.43	*	*	*	*	*
	PSS Ethyl acetate	-28.57	*	*	*	*	*
	PSS n-hexane	-42.86	*	*	*	*	*
Barks	**PSB Crude**	*	*	*	*	*	*
	PSB Butanol	*	*	*	*	*	*
	PSB Ethyl acetate	-42.86	-40	*	*	-77.78	*
	PSB n-hexane	*	*	*	*	*	*
Roots	**PSR Crude**	*	-60	*	*	*	*
	PSR Butanol	0.14	*	*	*	*	*
	PSR Ethyl acetate	-14.29	*	*	*	*	*
	PSR n-hexane	*	*	*	-77.78	-66.67	*

Key: *: Not applicable;ST: *Salmonella typhi;* EC: *Escherichia coli*; CA: *Candida albican*; AN: *Aspergillus niger*, TR: *Trichophyton rubrum*, MC: *Microsporon canis*, PSLCrude: *Pterocarpus santalinodes* Leaf (Crude extract); PSLButanol: *Pterocarpus santalinodes* Leaf (Butanol fraction); PSLEthyl acetate: *Pterocarpus santalinodes* Leaf (Ethyl acetate fraction); PSLn-hexane: *Pterocarpus santalinodes* Leaf (n-hexane fraction), PSSCrude: *Pterocarpus santalinodes* Seed (Crude extract); PSSButanol: *Pterocarpus santalinodes* Seed (Butanol fraction); PSSEthyl acetate: *Pterocarpus santalinodes* Seed (Ethyl acetate fraction); PSSn-hexane: *Pterocarpus santalinodes* Seed (n-hexane fraction), PSBCrude: *Pterocarpus santalinodes* Bark (Crude extract); PSBButanol: *Pterocarpus santalinodes* Bark (Butanol fraction); PSBEthyl acetate: *Pterocarpus santalinodes* Bark (Ethyl acetatefraction); PSBn-hexane: *Pterocarpus santalinodes* Bark (n-hexane fraction), PSRCrude: *Pterocarpus santalinodes* Root (Crude extract); PSRButanol: *Pterocarpus santalinodes* Root (Butanol fraction); PSREthyl acetate: *Pterocarpus santalinodes* Root (Ethyl acetate fraction); PSRn-hexane: *Pterocarpus santalinodes* Root (n-hexane fraction).

The determination of PIDG values for the test organisms against the plant extracts/fractions showed that crude methanol extract (28.57%) and ethyl-acetate fraction (0.14%) of the leaves, ~~Butanol~~ butanol fraction (0.14%) of the root (all against ~~*Salmonella*~~ *S. typhi*) were the most potent test samples using the samples that outstrips the positive control used, which was 5 µg/mL Ciprofloxacin was used as the threshold/standard. Additional information from further studies on the mechanism of action of these extracts and fractions might contribute to its usage as an alternative to the conventional antibacterial drugs for the management of ~~*Salmonella*~~ *S. typhi* and *E. coli* implicated diseases.

4. CONCLUSION

The study revealed that the various parts of the plant ~~has~~ have antibacterial potentials especially organisms implicated in diarrhea as was seen with *S. typhi* and *E. coli*. The plant can be said to have negligible antifungal activities. It also shows that the solvent used for extraction or fractionation can affect the antimicrobial effect of the extracts/fractions. There is a need for more studies towards isolation, identification, and characterization of the bioactive agents and also test for activities against wider spectrum of organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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