

# 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 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 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 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 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 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 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.

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

56  
57 In Nigeria many indigenous plants including *Pterocarpus Santalinoidea* are used as food or medicine. The  
58 tender leaves are used as vegetable in soup preparing, while the stem bark is used in making pepper  
59 soup. *Pterocarpus santalinoides* is a plant believed to possess potent antibacterial properties in ethno  
60 medicine. The plants are used in treating rheumatism, diarrhea, dysentery, cough, asthma, diabetes,  
61 malaria, elephantiasis, cold and others [7]. The use of the plant leaves in treating skin disease such as  
62 eczema, candidiasis, and acne have been reported [8]. The use of the concoction made from its root in  
63 treating asthmatic patients have also been reported [9]. It is also used in treating diarrhea which is a  
64 major cause of death as it has a proven anti enteropaling activity in traditional medicine [10].

65  
66 This research is aimed at investigating the antimicrobial activities of various parts of the plant against  
67 some indicative organisms implicated in their traditional use.

## 68 **2. MATERIALS AND METHODS**

### 69 **2.1 Plants Materials**

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72  
73 The fresh leaves, seeds, bark and roots of *Pterocarpus santalinoides* were sourced in September 2016  
74 from Okofia - Otolu Nnewi, Anambra state, Nigeria, latitude 5°58'48.86" and longitude 6°54'30.78". They  
75 were identified and authenticated by a Curator in the Department of Pharmacognosy, Faculty of  
76 Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria.

### 77 **2.2 Culture Media and Reagents**

78  
79  
80 Culture media used were Nutrient Broth, Mueller-Hinton agar (Oxoid Limited, England) and Sabouraud  
81 dextrose agar (Titan Biotech, India). Culture media were prepared according to the instructions of the  
82 manufacturers.

83  
84 Reagents used include McFarland 0.5 turbidity standard (prepared from Barium chloride, Sulfuric acid  
85 and water), sodium hypochlorite solution and dimethyl sulphoxide (Triveni Chemicals, India).

### 86 **2.3 Equipment**

87  
88  
89 Equitron partially automatic autoclave (Medica Instrument Manufacturing CO., India), hot air oven  
90 (Genlab, UK), incubator (Genlab, UK), electronic weighing balance (Ohaus Corp., USA).

### 91 **2.4 Microbial Test Organisms**

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93  
94 The microorganisms used in this study were two bacteria isolates (*Escherichia coli* and *Salmonella typhi*)  
95 stored at 4 – 8 °C in Mueller-Hinton Agar slants and four fungi isolates (*Candida albicans*, *Aspergillus*  
96 *niger*, *Trichophyton rubrum* and *Microsporon canis*). These were clinical isolates previously purified and  
97 standardized to McFarland. Their susceptibility to commonly used antibiotics was already established.  
98 The isolates were obtained from the Department of Pharmaceutical Microbiology & Biotechnology,  
99 Nnamdi Azikiwe University, Awka, Nigeria.

### 100 **2.5 Extraction of Plant Materials**

101  
102  
103 The different plant parts of *Pterocarpus santalinoides* were prepared using the method of [11]. They were  
104 dried at room temperature (25 °C), grounded into a fine powder using laboratory grinding mill and stored  
105 in a cool and dry place. Total extraction of each plant materials were prepared by mixing with solvent  
106 (methanol) in the ratio of 1:10 (plant material/solvents) in a cold maceration system. The plant materials

107 were soaked in 1000 ml solvent in conical flasks for 12 hours. The extracts were then filtered using  
108 Whatman No. 1 filter paper and solvents removed through evaporation under reduced pressure at 45 °C  
109 using a rotary evaporator (Yamato, USA). The extracts were kept in stoppered sample vials at 4 °C until  
110 they were used.

## 111 **2.6 Fractionation of Crude Methanol Extracts**

112  
113  
114 The crude methanol extracts of the different parts of the plant were fractionated using solvent-solvent  
115 method with n-hexane, ethyl acetate and butanol in order of increasing polarity.

## 116 **2.7 Percentage Yield Determination**

117  
118  
119 The percentage yield (% w/w) from all the dried crude methanol extracts was calculated using the  
120 formula outlined below:

$$121 \quad \text{Percentage (\% ) yield} = \frac{\text{Weight of extract}}{\text{Weight of dried plant material}} \times 100 \dots \dots \dots \text{Equation}$$

122 1

## 123 124 125 **2.8 Preliminary Antimicrobial Assay**

126  
127  
128 The Preliminary antimicrobial assay for each of the crude extracts and their respective fractions was  
129 carried out using the agar well diffusion assay as described by [12]. The antimicrobial activity of the plant  
130 parts (i.e. crude extracts and fractions) were tested against standard clinical isolates (two bacteria and  
131 four fungi isolates) as listed above. A 0.5 McFarland standard bacterial/fungal suspension of each of the  
132 test isolates were prepared and these formed both the bacterial and fungal stock solutions used in the  
133 agar well diffusion assays as outlined below.

## 134 135 **2.9 Agar Well Diffusion Assay**

136  
137 Briefly, the media, Mueller-Hinton agar, MHA (Oxoid, USA) and Sabouraud dextrose Agar, SDA, were  
138 prepared and treated according to the manufacturer's specification. MHA (38 g) and SDA (65 g)  
139 respectively were mixed with 1L of sterile distilled water and sterilized at 121 °C for 15 mins. The media  
140 was allowed to cool to 50 °C and later transferred into 90 mm sterile agar plates and left to set. The  
141 sterile MHA and SDA plates were inoculated with the test culture from each of the test suspensions,  
142 thereafter, 20 ml of the sterile molten agar cooled to 50 °C was added then to the plate and was rocked  
143 clockwise and anti-clockwise to ensure even distribution of the test organism. This was done to obtain  
144 uniformity of the inoculums. A sterile cork borer was used to make wells (6 mm in diameter) on each of  
145 the MHA and SDA plates respectively. Aliquots of 60 µl of the stock concentration (100 mg/ml) of each  
146 extracts and fractions, reconstituted in dimethyl sulphoxide (DMSO) were applied in each of the wells in  
147 the culture plates previously seeded with the test organisms. Ciprofloxacin (5 µg) and miconazole (50  
148 µg/ml) served as the positive controls for the bacteria and fungi respectively, while DMSO served as the  
149 negative control. The cultures were incubated at 37 °C for 18 - 24 hours for the bacterial plates and 25 –  
150 27 °C for 48 hours for the fungal plates respectively. The antimicrobial potential for each extract and  
151 fraction was determined by measuring the zone of inhibition around each well (excluding the diameter of  
152 the well). For each extract and fraction, three replicates were conducted against each organism. Each  
153 extract and fraction was tested against all the bacterial and fungal isolates.

154  
155 Furthermore, based on the recorded activity, the extracts and fractions that showed activity were  
156 subjected to minimum inhibitory concentration (MIC) and maximum biocidal concentration (MBC)  
157 Determinations.

## 158 159 **2.10 Anti-dermatophyte Activity**

160  
161 The anti-dermatophyte activity was conducted using the method as described by [13]. A sterile swab was  
162 used to aseptically inoculate each of the fungal suspension (*Trichophyton rubrum* and *microsporon canis*)

163 on the surface of sterilized Sabouraud dextrose agar. The tests were carried out using a stock  
164 concentration of 100 mg/ml prepared by dissolving 200 mg of the crude extracts and fractions into 2 ml of  
165 DMSO. A well of 6 mm diameter was made in the agar plate then loaded with 60 µl of each of the stock  
166 concentration of the crude extracts and fractions of the test samples and incubated at 28 ± 2 °C for 15 -  
167 20 days. The inhibition zone was observed and then recorded in millimeters using a transparent metre  
168 rule. The test was conducted in triplicate and results presented as mean. Miconazole 50 µg/ml served as  
169 the standard positive control against the *dermatophytic species*.  
170

### 171 **2.11 Determination of Minimum Inhibitory Concentration (MIC)**

172  
173 The MIC was interpreted as the lowest concentration of the test samples (extracts and fractions) that  
174 inhibited visible growth. The MICs of the active samples were determined by agar dilution method as  
175 described by [13, 14] for the antimicrobial and anti-dermatophytic activities respectively. The MIC was  
176 determined for the micro-organisms that showed reasonable sensitivity to the test crude extracts and  
177 fractions. In this test, a stock solution of the crude extracts and fractions (2,000 mg/ml) was made, then 2-  
178 fold serial dilution was done to get graded dilutions (1000, 500, 250, 125, and 62.5 mg/ml) of each of the  
179 crude extract and fraction. Then 1 ml of each of these concentration was transferred into a sterile petri  
180 dish and properly mixed with 9 ml of molten Mueller-Hinton agar and Sabouraud dextrose agar, then  
181 cooled to 45 - 50 °C. After the mixing was done the final concentrations becomes 100, 50, 25, 12.5, and  
182 6.25 mg/ml respectively. Finally, the different test organisms were streaked on the solidified agar properly  
183 labeled and incubated at 37 °C for bacteria and 28 ± 2 °C for fungi. The bacteria where incubated for 18 –  
184 24 hours, while *Candida albicans* and *Aspergillus niger* were incubated for 48 hours and the  
185 dermatophytes incubated for 15 - 20 days. Each experiment was performed in triplicate.  
186

### 187 **2.12 Determination of the Minimal Bactericidal/Fungicidal Concentration of the Crude** 188 **Extracts and Fractions (MBC)**

189  
190 This is the minimal concentration which kills off the cells in a microbial population. The MBC/MFC is the  
191 lowest concentration producing no evidence of growth [14]. It is an extension of the MIC procedure  
192 (stated above) carried out. Here, the plates that show no visible growth in the MIC test were selected.  
193 Then, they are incubated for 48 more hours. Thereafter, the plates were examined for signs of microbial  
194 growth.  
195

### 196 **2.13 Determination of the Percentage Inhibition Diameter Growth (PIDG)**

197  
198 Following the observation for the antimicrobial evaluation, the percentage inhibition diameter growth  
199 (PIDG) values were determined according to the equation as below.  
200

$$201 \text{PIDG (\%)} = \frac{\text{Diameter of sample} - \text{Diameter of control}}{\text{Diameter of control}} \times 100 \dots \dots \dots \text{equation}$$

202  
203  
204  
205

### 206 **2.14 Data Analysis**

207  
208 The results were expressed as mean. Statistical analysis was carried out using one way analysis of  
209 variance (ANOVA) and SPSS (version 20) statistical program. The obtained results were considered  
210 significant at P = 0.05.  
211

## 212 **3. RESULTS**

### 213 **3.1 Extraction, Fractionation and Percentage Yield**

214  
215 Each of the leaves, seeds, barks and roots of *Pterocarpus santalinoides* were extracted with methanol as  
216 primary solvent. This yielded four (4) crude methanol extracts of the plant samples. The leaves (8.27 %)  
217

218 gave the highest percentage yield as depicted in (Table 1). This was followed by the bark (5.97 %). The  
 219 least value was observed with the seeds (1.97 %).

220  
 221 The solvent-solvent fractionation of the four (4) crude methanol extract of the plant, *Pterocarpus*  
 222 *santalinoides* with n-hexane, ethyl acetate and butanol in their order of increasing polarity gave a total of  
 223 four (4) fractions each of the three (3) secondary solvents giving a total sample of sixteen ((4) each of the  
 224 crude methanol extracts, n-hexane, ethyl acetate and butanol fractions respectively) samples.

225  
 226 **Table 1: The percentage yield of crude extracts obtained from *Pterocarpus santalinoides***  
 227

Plant Parts	Powdered Plant materials (Wt. in g)	Plant 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

228  
 229  
 230 **3.2 Antimicrobial Activity of *Pterocarpus santalinoides* Extracts and Fractions against**  
 231 **the Test Isolates**  
 232

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

238  
 239 A total of 16 crude methanol extracts and fractions of the plant parts were tested for their antimicrobial  
 240 activity among which 12 samples showed inhibitory activity on at least one or more of the test pathogenic  
 241 microorganisms. In contrast, 4 samples (PSL n-hexane, PSB Crude, PSB Butanol, PSB n-hexane)  
 242 showed no activity against any of the selected microorganism at the tested concentration. The result  
 243 showed that ethyl acetate fraction of the plant bark (PSB Ethyl acetate) has antimicrobial activity against  
 244 3 of the tested organisms (*Salmonella typhi*, *E. coli* and *Microsporon canis*).

245  
 246 The Ciprofloxacin (5 µg/ml) conventional drug used as control was active against the bacterial isolates  
 247 with mean inhibition zone diameter (IZD) of 7 mm against *S. typhi* and 5 mm against *E. coli*. The  
 248 Miconazole (50 µg/ml) used as control for fungal isolates was active against all the fungal isolates with  
 249 mean inhibition zone diameter (IZD) of 18 mm for *Candida albicans* and *Microsporon canis* and 9 mm for  
 250 *Aspergillus niger* and *Trichophyton rubrum* respectively. This reveals that the tests organisms used in the  
 251 course of this work were susceptible strains. The DMSO serving as the negative control had no inhibition  
 252 activity on any of the test organisms. The results of the antimicrobial activity of the plant parts are  
 253 summarized in Tables 2.

254 **Table 2: Mean Inhibition Zone Diameter (IZD) mm of the preliminary antimicrobial activity of the**  
 255 **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

<b>Seeds</b>	<b>PSS Crude</b>	0	5	0	0	0	0
	<b>PSS Butanol</b>	2	0	0	0	0	0
	<b>PSS Ethyl acetate</b>	5	0	0	0	0	0
	<b>PSS n-hexane</b>	4	0	0	0	0	0
<b>Barks</b>	<b>PSB Crude</b>	0	0	0	0	0	0
	<b>PSB Butanol</b>	0	0	0	0	0	0
	<b>PSB Ethyl acetate</b>	4	3	0	0	4	0
	<b>PSB n-hexane</b>	0	0	0	0	0	0
<b>Roots</b>	<b>PSR Crude</b>	0	2	0	0	0	0
	<b>PSR Butanol</b>	7	0	0	0	0	0
	<b>PSR Ethyl acetate</b>	6	0	0	0	0	0
	<b>PSR n-hexane</b>	0	0	0	2	6	0
<b>Control</b>	<b>Ciprofloxacin 5 µg/ml</b>	7	5	nd	nd	nd	nd
	<b>Miconazole 50 µg/ml</b>	Nd	Nd	18	9	18	9
	<b>DMSO</b>	0	0	0	0	0	0

256 **Key:** nd: not determined; ST: *Salmonella typhi*; EC: *Escherichia coli*; CA: *Candida albican*; AN:  
257 *Aspergillus niger*, MC: *Microsporion canis*, TR: *Trichophyton rubrum*, PSLCrude: *Pterocarpus santalinodes*  
258 Leaf (Crude extract); PSLButanol: *Pterocarpus santalinodes* Leaf (Butanol fraction); PSEthyl acetate:  
259 *Pterocarpus santalinodes* Leaf (Ethyl acetate fraction); PSLn-hexane: *Pterocarpus santalinodes* Leaf (n-  
260 hexane fraction), PSSCrude: *Pterocarpus santalinodes* Seed (Crude extract); PSSButanol: *Pterocarpus*  
261 *santalinodes* Seed (Butanol fraction); PSSEthyl acetate: *Pterocarpus santalinodes* Seed (Ethyl acetate  
262 fraction); PSSn-hexane: *Pterocarpus santalinodes* Seed (n-hexane fraction), PSBCrude: *Pterocarpus*  
263 *santalinodes* Bark (Crude extract); PSBButanol: *Pterocarpus santalinodes* Bark (Butanol fraction);  
264 PSBEthyl acetate: *Pterocarpus santalinodes* Bark (Ethyl acetate fraction); PSBn-hexane: *Pterocarpus*  
265 *santalinodes* Bark (n-hexane fraction), PSRCrude: *Pterocarpus santalinodes* Root (Crude extract);  
266 PSRButanol: *Pterocarpus santalinodes* Root (Butanol fraction); PSREthyl acetate: *Pterocarpus*  
267 *santalinodes* Root (Ethyl acetate fraction); PSRn-hexane: *Pterocarpus santalinodes* Root (n-hexane  
268 fraction); Standard error: ST: 0.7.  
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270  
271  
272  
273 The comparison of the activity of the extracts and fractions of *Pterocarpus santalinoides* according to the  
274 parts showed that the extracts and fractions significantly ( $p=0.05$ ) inhibited some of the test organisms.  
275 For the leaves; the crude methanol extract (PSL Crude) had better antimicrobial activity to the only  
276 sensitive organism which is *Salmonella typhi* followed by ethyl acetate fraction (PSL Ethyl acetate) and  
277 Butanol fraction (PSL Butanol) respectively. The n-hexane fraction (PSL n-hexane) had no inhibition  
278 activity. This result indicates that the active agents in the plant leaves against this organism are chiefly  
279 polar compounds. For the Seeds; the ethyl acetate fraction (PSS Ethyl acetate) had the best inhibition  
280 activity against *S. typhi* followed by n-hexane (PSS n-hexane) and butanol (PSS Butanol) fractions  
281 respectively. The crude methanol extract (PSS Crude) was not active against *S. typhi*, however, was the  
282 only sample of the plant seeds active against *E. coli*. No part of the plant seeds has inhibition activity  
283 against *C. albicans*, *A. niger*, *M. canis* and *T. rubrum*. From this result, the phytochemicals in the seeds  
284 active against *S. typhi* are mainly non-polar compounds, however, the compounds active against *E. coli*  
285 are polar compounds. It also showed that after fractionation of the crude methanol extract (PSS Crude),

286 the active principles in the seed against *E. coli* was lost, which indicates an antagonistic effect of the  
287 fractionation process on the active principles. No part of the bark had inhibition activity on any of the  
288 tested organisms except for ethyl acetate fraction (PSB ethyl acetate) which had activity against *S. typhi*,  
289 *E. coli* and *M. canis*. This result showed that the active principles in the plant are bipolar compounds. This  
290 is logically in accordance to some work done [15] that tested the antimicrobial activities of the ethanol and  
291 water extracts of *P. santalinoides* bark against organisms including *S. typhi* and *E. coli*, of which the water  
292 extracts (a polar solvent) did not inhibit these test isolates at the concentration used. The ethanol extracts  
293 only marginally inhibited the organisms. For the roots, the crude methanol extract (PSR Crude) and the n-  
294 hexane fraction (PSR n-hexane) had no activity against *S. typhi*. The butanol fraction (PSR Butanol) had  
295 the best activity against *S. typhi*. Only the crude methanol extract (PSR Crude) showed inhibition activity  
296 against *E. coli*. The best antimicrobial activity was observed with the n-hexane fraction (PSR n-hexane).

297  
298 Comparison of the susceptibility of all the test organisms to the various extracts/fractions of the plant  
299 parts, indicated that *Salmonella typhi* appeared to be the most sensitive organism to metabolites from  
300 *Pterocarpus santalinoides* with 9 of the extracts/fractions showing inhibitory activity and the best activity  
301 was observed with the crude methanol extract of the plant leaves (PSL Crude) were IZD of 8 mm, MIC of  
302 50 mg/ml and MBC of 100 mg/ml were recorded. In contrast, *Candida albicans* and *Trichophyton rubrum*  
303 were found to be the most resistant organisms where none of the extracts/fraction showed antimicrobial  
304 effect at tested concentrations.

305  
306 Three samples (PSS Crude, PSB Ethyl acetate and PSR Crude) showed antimicrobial activities against  
307 *Escherichia coli* with the best activity observed for crude methanol extract of the plant seed (PSS Crude)  
308 where IZD of 5 mm and MIC of 100 mg/ml were record. No part of the plants' leaves extract/fractions had  
309 activity against *E. coli*. This is similar to work done by [16], who evaluated the antimicrobial activities of  
310 the methanol extracts of the plant leaves against *S. aureus* and *E coli*, of which *E. coli* showed no  
311 activity at all concentrations (up-to a maximum concentration of 50 mg/ml). *Microsporon canis* was  
312 inhibited by only two fractions (PSB Ethyl acetate and PSR n-hexane), while *Aspergillus niger* was  
313 inhibited by only one fraction (PSR n-hexane).

314  
315 The ethyl acetate fraction of the plant bark (PSB Ethyl acetate) showed activities against 3 of the tested  
316 isolates producing IZD of 4 mm against *Salmonella typhi*, 3 mm against *Escherichia coli* and 4 mm  
317 against *Microsporon canis* showing an extended activity than the other extracts/fractions with activities  
318 against 2 Gram negative and 1 fungi isolates. This is similar to a work done by [17], were a broad  
319 spectrum activity of the plant bark was reported as was observed in this study. However, the best  
320 antifungal activity was observed for n-hexane fraction of the plant root (PSR n-hexane) where an IZD of 2  
321 mm against *Aspergillus niger* and 6 mm against *Microsporon canis* were recorded.

322  
323 The result of this study showed that the various extracts and fractions of the plant have antimicrobial  
324 activity against *Salmonella typhi*. Similarly, [18] reported the activity of this plant to this organism.

### 325 326 **3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal** 327 **Concentration (MBC/MFC) of *Pterocarpus santalinoides* Extracts and Fractions** 328

329 The lowest concentration of the extracts and fractions of the plant parts at which no growth of  
330 microorganism was observed upon visual observation after incubation was considered the MIC value.  
331 The range of MIC of the plants extracts and fractions recorded was 12.5 – 100 mg/ml. The n-hexane  
332 fraction of the root gave the lowest MIC value (12.5 mg/ml) against *Microsporon canis*, followed by ethyl  
333 acetate fraction of the bark with 25 mg/ml against *E. coli* and *Microsporon canis*.

334  
335 The lowest concentration of the extracts and fractions of the plant parts at which no growth of  
336 microorganism was observed upon visual observation after further incubation of the MIC plates were  
337 recorded as the Minimal Bactericidal Concentration (MBC) for bacteria isolates and Minimum Fungicidal  
338 Concentration (MFC) for fungal isolates. This is shown in Table 4. The ethyl acetate fraction of the bark

339 (PSBE) and the n-hexane fraction of the plant root (PSRn) gave the lowest value (25 mg/ml) against *E.*  
340 *coli* and *Microsporon canis* respectively.

341

### 342 **3.4 Determination of the Percentage Inhibition of Diameter Growth (PIDG)**

343

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

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UNDER PEER REVIEW



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

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

364  
 365 **Key:** ST: *Salmonella typhi*; EC: *Escherichia coli*; CA: *Candida albicans*; AN: *Aspergillus niger*, MC: *Microsporion canis*, TR: *Trichophyton rubrum*,  
 366 PSLC: *Pterocarpus santalinoides* Leaf (Crude extract); PSLB: *Pterocarpus santalinoides* Leaf (Butanol fraction); PSLE: *Pterocarpus santalinoides*  
 367 Leaf (Ethyl acetate fraction); PSLn: *Pterocarpus santalinoides* Leaf (n-hexane fraction), PSSC: *Pterocarpus santalinoides* Seed (Crude extract);  
 368 PSSB: *Pterocarpus santalinoides* Seed (Butanol fraction); PSSE: *Pterocarpus santalinoides* Seed (Ethyl acetate fraction); PSSn-hexane:  
 369 *Pterocarpus santalinoides* Seed (n-hexane fraction), PSBCrude: *Pterocarpus santalinoides* Bark (Crude extract); PSBB: *Pterocarpus santalinoides*  
 370 Bark (Butanol fraction); PSBE: *Pterocarpus santalinoides* Bark (Ethyl acetate fraction); PSBn: *Pterocarpus santalinoides* Bark (n-hexane fraction),  
 371 PSRC: *Pterocarpus santalinoides* Root (Crude extract); PSRB: *Pterocarpus santalinoides* Root (Butanol fraction); PSRE: *Pterocarpus santalinoides*  
 372 Root (Ethyl acetate fraction); PSRn: *Pterocarpus santalinoides* Root (n-hexane fraction).  
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378 **Table 4: Minimum Bactericidal/Fungicidal Concentration (mg/ml) of the crude extracts and fractions of *Pterocarpus santalinoides***  
 379 **against tests isolates**

380 Isolate	PSLC	PSLB	PSLE	PSLn	PSSC	PSSB	PSSE	PSSn	PSBC	PSBB	PSBE	PSBn	PSRC	PSRB	PSRE	PSRn
381 ST	100	100	100	-	-	-	50	100	-	-	-	-	-	-	100	-
382 EC	100	-	-	-	-	-	-	-	-	-	25	-	-	-	-	-
383 CA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
384 AN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
385 TR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
386 MC	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-	25

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

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

407  
408 The Research conducted by [6], on the preliminary phytochemical analysis of methanolic extract of  
409 *Pterocarpus santalinoides* leaves showed the presence of alkaloids, anthocyanins, carotenoids,  
410 flavonoids, resins, saponins, steroids, terpenoids and tannins, while [19] revealed the presence of  
411 leucoanthocyanins, coumarins, flavonoids, mucilage, saponins and tanins. The presence of alkaloids,  
412 flavonoids, tannins, saonins, phenolics and cyanogenic glycosides were revealed by [15] in the ethanoloc  
413 extracts of the plant stem bark. No study was seen on the phytoconstituents of the plant's seed and root,  
414 but relatively will have the compounds as listed above, though environmental factors affects plants  
415 phytoconstituents. The antibacterial activities observed with these plant extracts and samples can be  
416 attributed to these secondary metabolites [15, 20]

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

450 **Key:** \*: Not applicable; ST: *Salmonella typhi*; EC: *Escherichia coli*; CA: *Candida albican*; AN: *Aspergillus*  
 451 *niger*, TR: *Trichophyton rubrum*, MC: *Microsporon canis*, PSLCrude: *Pterocarpus santalinodes* Leaf (Crude  
 452 extract); PSLButanol: *Pterocarpus santalinodes* Leaf (Butanol fraction); PSLEthyl acetate: *Pterocarpus*  
 453 *santalinodes* Leaf (Ethyl acetate fraction); PSLn-hexane: *Pterocarpus santalinodes* Leaf (n-hexane  
 454 fraction), PSSCrude: *Pterocarpus santalinodes* Seed (Crude extract); PSSButanol: *Pterocarpus*  
 455 *santalinodes* Seed (Butanol fraction); PSSEthyl acetate: *Pterocarpus santalinodes* Seed (Ethyl acetate  
 456 fraction); PSSn-hexane: *Pterocarpus santalinodes* Seed (n-hexane fraction), PSBCrude: *Pterocarpus*  
 457 *santalinodes* Bark (Crude extract); PSBButanol: *Pterocarpus santalinodes* Bark (Butanol fraction);  
 458 PSBEthyl acetate: *Pterocarpus santalinodes* Bark (Ethyl acetate fraction); PSBn-hexane: *Pterocarpus*  
 459 *santalinodes* Bark (n-hexane fraction), PSRCrude: *Pterocarpus santalinodes* Root (Crude extract);  
 460 PSRButanol: *Pterocarpus santalinodes* Root (Butanol fraction); PSREthyl acetate: *Pterocarpus*  
 461 *santalinodes* Root (Ethyl acetate fraction); PSRn-hexane: *Pterocarpus santalinodes* Root (n-hexane  
 462 fraction).  
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472 The determination of PIDG values for the test organisms against the plant extracts/fractions showed that  
473 crude methanol extract (28.57%) and ethyl-acetate fraction (0.14%) of the leaves, Butanol fraction  
474 (0.14%) of the root (all against *Salmonella typhi*) were the most potent test samples using the samples  
475 that outstrips the positive control used, which was 5µg/mL Ciprofloxacin as the threshold/standard.  
476 Additional information from further studies on the mechanism of action of these extracts and fractions  
477 might contribute to its usage as an alternative to the conventional antibacterial drugs for the management  
478 of *Salmonella typhi* and *E. coli* implicated diseases.

479

#### 480 4. CONCLUSION

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

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#### 490 COMPETING INTERESTS

491

492 Authors have declared that no competing interests exist.

493

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