

Studies on the Antibacterial Profile of *Bryocarpus coccineus* and *Zanthoxylum piperitum*

ABSTRACT

The antibacterial efficacy of methanolic leaf extracts of *Bryocarpus coccineus* and *Zanthoxylum piperitum* was determined against six clinical isolates and three typed cultures respectively. The percentage yield of the extracts was calculated, and it showed 5.6% for *Bryocarpus coccineus* and 4% for *Zanthoxylum piperitum*. Preliminary phytochemical screening of the two extracts showed the presence of saponins, steroids, glycosides, flavonoids and resin. The extracts effectively inhibited the growth of *Escherichia coli* and *Bacillus subtilis* at different concentrations. The extract of *B. coccineus* inhibited *S. aureus*, *K. pneumonia* and *P. aeruginosa* at different concentration, while that of *Z. piperitum* inhibited *S. aureus* and *P. aeruginosa* at different concentration. *B. coccineus* extract had its MIC at 6.25mg/ml against clinical isolate of *Escherichia coli* and *Bacillus subtilis*. *Z. piperitum* methanolic extract had its MIC at 6.25 mg/ml against clinical isolate *Bacillus subtilis*. All the plants extract had no activity against *Salmonella typhi*, and *B. coccineus* had no activity against *Klebsiella pneumonia*. The results of statistical analysis (ANOVA) of the *B. coccineus* showed that F-cal. is greater than F-tab. This means there is significant difference on the activity of the extract, while that of *Z. piperitum* showed that F-cal is less than F-tab. meaning there is no significant difference on the activity of the extract. This reveals the importance of leaf extracts of *B. coccineus* and *Z. piperitum* in the control of resistant microbial strains.

KEYWORDS: *Bryocarpus coccineus*, *Zanthoxylum piperitum*, Minimum Inhibitory Concentration (MIC), Antibacterial Activity

1. INTRODUCTION

25 Knowledge of the chemical constituents of plant is important for the discovery of therapeutic agents. Medicinal
26 plants contain physiological bioactive phytochemicals that over the years have been used in traditional medicine [1,
27 2]. Gills [3], reported that plants contain a wide variety of bioactive phytochemicals. Globally, plant extracts are
28 employed for their antimicrobial, antiviral and antifungal properties. A number of plants including *Brysocarpus*
29 *coccineus* and *Zanthoxylum piperitum* have been used in traditional medicine for many years due to their
30 antimicrobial properties. They have been found to possess inhibitory and bactericidal effect on most microbes [3, 4,
31 5, 6, 7, and 8]

32 *Brysocarpus coccineus* is a climbing shrub found in Africa, the plant especially the leaves is used in traditional
33 medicine for the treatment of venereal diseases, impotency, diarrhoea, jaundice, piles, dysentery, ear ache, sore
34 mouth, tumour, wounds, stomatitis, rheumatism, swelling and urinary disorders [9]. The pharmacological properties
35 of *Brysocarpus coccineus* as an antioxidant, anti-inflammatory, analgesic, antidiarrheal and antipyretic have been
36 established [9, 10, 11, and 12]. The presence of Quercetin 3-O-B-D- glucose from the bioactive ethyl acetate and n-
37 butanol soluble parts of ethanol extracts of *Brysocarpus coccineus* was also established [13]. It has traditional names
38 such as amuje, wewa and ade, while the common name is crimson. It is very common in old farmlands and open
39 places in the forest.

40 *Zanthoxylum piperitum* is a small shrub growing to 2meters, native to the Himalayan and mountainous region of
41 China, Korea, Manchuria and Japan, with brownish prickly bark and paired spines. *Zanthoxylum piperitum* have
42 been found to possess antibacterial, antifungal, carminative, diuretic, parasiticide and stimulant effect [14]. Its
43 beneficial effects have been traditionally associated with antibacterial, anti-lipid peroxidative and antiviral activities
44 [15]. The resin contained in the bark, and especially in that of the roots, is powerfully stimulant and tonic. It has
45 common names such as Japanese called it sansho (mountain pepper) and its English name is Japanese pepper.

46 2. MATERIALS AND METHODS

47 2.1. Collection and Identification of Plant Samples

48 The plant material used for the various test in this work is *Zanthoxylum piperitum* and *Brysocarpus coccineus*. The
49 leaves were obtained from different locations in Nsukka. *Brysocarpus coccineus* was got from an open farm-land in

50 Ehandiagu area, and *Zanthoxylum piperitum* was got from a thick forest in Orba area (all in Nsukka). The leaves
51 were identified by Mr Ozioko from Bioresource Development and Conservation Programme, Nsukka. These plants
52 were then air-dried for about 5 days. The dried parts were subsequently reduced to fine powder using an electric
53 grinder.

54 **2.2. Methanol extraction of anti-microbial substances from *Bryocarpus coccineus* and *Zanthoxylum*** 55 ***piperitum* leaves.**

56 A 25g weight of the ground leaves was measured into sterile 500ml bottles. Then 250ml of the methanol solvent
57 was added. All bottles were stoppered to avoid loss by evaporation. The soaked leaves were then sieved using a
58 sterile morcelain cloth and finally with No. 1 Whatman filter paper. The filtrates were collected and allowed to
59 evaporate. The concentrated extracts were scooped into sterile bottles, labelled accordingly and stored in the
60 refrigerator while the research lasted.

61 **2.3. Percentage Yield of Extract**

62 The percentage yield of the extracts was determined using the formula below;

63 Percentage yield (% yield) =
$$\frac{\text{mass of dried extract (g)}}{\text{Mass of pulverized plant material (g)}} \times 100 \dots \dots (1)$$

64 **2.4. Phytochemical Analysis**

65 Powdered samples were subjected to phytochemical analysis to screen for active constituents in the leaves of
66 *Bryocarpus coccineus* and root bark of *Zanthoxylum piperitum* using standard procedures of analysis [16]. Tests
67 were done to detect the presence of alkaloids, tannins, saponins, resins, flavonoids, steroids, Glycosides, terpenoids,
68 Carbohydrate and Resins.

69 **2.4.1. Test for Alkaloids**

70 Exactly 0.1 gram of ground sample was boiled with 5 ml of 2% hydrochloric acid on a steam bath. This was filtered
71 and 1ml portion of the filtrate treated with 2 drops of the following reagents, and the results were recorded;

- 72 • Mayers reagent (Potassium mercuric iodide solution) and observed for cream colored precipitate.
- 73 • Dragendroff's reagent (Bismuth Potassium Iodide solution) and observed for precipitation.

74 **2.4.2. Test for Tannins:**

75 A 0.1 gram of the ground sample was boiled with 5 ml of 45% ethanol for 5 minutes, cooled and filtered. About 1ml
76 of the filtrate was diluted in water and few drops of ferric solution was added and observed for a transient greenish
77 to black colour.

78 **2.4.3. Test for Flavonoids**

79 Exactly 0.2 gram of ground sample was heated with 10ml of ethyl acetate in boiling water for 1 minute. This was
80 filtered and the filtrate used for the following tests, and results recorded;

- 81 • About 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and observed for light
82 yellow coloration in ethyl acetate layer.
- 83 • Another 4 ml of the filtrate was shaken with 1 ml of dilute ammonia. The layers were allowed to separate
84 and the colour of the ammonia layer was observed for yellow coloration.

85 **2.4.4. Test for Saponins**

86 Exactly 0.1 gram of the ground sample was boiled with 5 ml of distilled water for 5 minutes and decanted while still
87 hot. The filtrate was used for the following test:

- 88 • Frothing test: 1 ml of the filtrate was diluted with 4 ml of distilled water shaken vigorously and observed on
89 standing for stable froth.
- 90 • Emulsion test: To 1ml of the filtrate was added 2 drops of olive oil, the solution was shaken and observed
91 for formation of emulsion.

92

93 **2.4.5. Test for Glycosides**

94 Briefly, 2 gram of the ground sample was added to 30 ml of water. The solution was heated on a water bath for 5
95 minutes, filtered and used for the following test:

- 96 • Exactly 5 ml of the filtrate was added to 0.2 ml of Fehling's solution A and Fehling's B solution until it turn
97 alkaline (tested with litmus paper). This was heated on a water bath for 2 minutes. The precipitate obtained
98 was observed for brick red coloration.
- 99 • Using 15 ml of dilute sulphuric acid instead of water, the above process was repeated and the quantity of
100 precipitate formed was observed and compared with that of the former experiment.

101 **2.4.6. Test for Steroids**

102 Briefly, 2 ml of acetic anhydride were added to 5 ml of sample of each plant. The sulphuric acid was added along
103 the side of the tubes and observed for a colour change from violent blue or green.

104 **2.4.7. Test for Terpenoids**

105 A quantity of 5 ml of methanol extract of plant sample was dissolved in 2 ml of chloroform. Concentrated sulphuric
106 acid were carefully added to form a layer and observed for a reddish brown coloration at the interface.

107 **2.4.8. Test for Carbohydrates**

108 Briefly, 0.1g of each sample was shaken vigorously with water and filtered, to the aqueous filtrate, was added few
109 drops of Molisch reagent followed by vigorous shaking again. Then 1ml of concentrated sulphuric acid was
110 carefully added down the side of the test tube to form a layer below the aqueous solution. A brown ring at the
111 interface indicates the presence of carbohydrates.

112 **2.4.9. Test for Resins**

113 Exactly 0.2g of the sample was extracted with 115ml of 96% ethanol. The samples each were poured into 20ml of
114 distilled water in a beaker. A precipitate occurring indicates the presence of resin.

115

116 **2.5. SCREENING FOR ANTIMICROBIAL ACTIVITY**

117 **2.5.1. Test Microorganisms**

118 The Microorganisms used in the test comprise a total of six bacterial isolates (*Pseudomonas aeruginosa*, *Bacillus*
119 *subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi* and *Staphylococcus aureus*) and type culture
120 isolates (*Staphylococcus aureus* (ATCC 2278), *Escherichia coli* (ATCC 2127), and *Pseudomonas aeruginosa*
121 (UCH 2078). The Clinical isolates were obtained from the laboratory stock of the Department of Pharmaceutical
122 Microbiology, University of Nigeria, Nsukka, while the Type isolates were obtained from the Bioresource
123 Development and Conservation Programme, Nsukka in Enugu State.

124 **2.5.2. Preparation of 0.5 McFarland Standard**

125 Briefly, 1.175g of Barium chloride crystal (BaCl_2) were weighed out and dissolved in 100ml distilled water to give
126 1.175% barium chloride solution ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$). A 1ml of concentrated tetraoxosulphate IV acid (H_2SO_4) was added
127 to 99ml of distilled water to make 1% H_2SO_4 solution. Furthermore, 9.95ml of 1% H_2SO_4 solution was mixed with
128 0.05ml of 1.175% Barium chloride solution to make 0.5 McFarland's standard. This was used to standardize the test
129 isolates.

130 **2.5.3. Preliminary Screening for the Antibacterial Activities of the extracts using the Agar Diffusion** 131 **Technique.**

132 Exactly 500mg of the extracts was dissolved in 5ml of the diluting solvent (Dimethylsulphoxide), to give a
133 concentration of 100 mg/ml, which served as the stock solution. A two-fold serial dilution of the stock solution was
134 carried out to obtain the following concentrations: 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. Approximately
135 0.2 ml of each dilution of the extract was introduced into wells bored onto Muller Hinton agar with a 6mm cork
136 borer. This was left to stand to allow the extract diffuse completely into the agar, and the plates were incubated at
137 37°C for 24 hours. The zones of inhibition were observed and recorded. The experiment was done in replicates, and
138 control, using antibiotics disc (Ciprofloxacin).

140 **2.5.4. Determination of the Minimum Inhibitory Concentration (MIC) using the Broth Micro dilution** 141 **Technique**

142 Approximately 5ml of Muller Hinton broth were pipetted into six tubes for each organism. A 0.5ml of the diluted
143 extract was added to the first tube and twofold serial dilution was done to get different concentration. A loopful of
144 the test isolates were inoculated into the various tubes using a sterile loop-full picked from an 18hrs old broth
145 culture of the organism, and incubated for 24hrs at 37⁰ C. The least dilution of extract that showed no activity, when
146 compared to the control, was taken as the minimum inhibitory concentration (MIC).

147

148 2.6. STATISTICAL ANALYSIS

149 The statistical analysis was done using one-way analysis of variance (ANOVA) to compare the susceptibility of the
150 test organisms to the extracts respectively, and determine the level of significance using the Fisher's Least
151 Significance Difference (F-LSD), all at 95% significance level.

152 3.0. RESULTS

153 Percentage Yield

154 The percentage yield of the methanol extract, (as shown in Table 1), revealed that *Brysocarpus coccineus* had the
155 highest percentage yield of 5.6% and 4.0% for *Zanthoxylum piperitum*.

156

157 **TABLE 1: Percentage Yields of Extracts**

158

| 159 Methanol extracts | 160 % Yields |
|-------------------------|--------------|
| 161 <i>B. coccineus</i> | 162 5.6 |
| 163 <i>Z. piperitum</i> | 164 4.0 |

161

162

163

164

165 Phytochemical Analysis

166 From the phytochemical analysis of the extracts, methanol extract contained high concentrations of Saponins and
167 Flavonoids; while Alkaloids, Tannin, Glycoside, Fats and Oil, and reducing sugar were found in medium
168 concentration as shown in Table 2.

169 **TABLE 2: Phytochemical Components of methanol extract of *Bryocarpus coccineus* and *Zanthoxylum***
170 ***piperitum*.**

| | <i>B. coccineus</i> | <i>Z. piperitum</i> | |
|-----|---------------------|---------------------|----|
| 171 | | | |
| 172 | Alkaloid | - | ++ |
| 173 | Saponins | +++ | + |
| 174 | Tannins | - | ++ |
| 175 | Glycosides | ++ | ++ |
| 176 | Flavonoids | ++ | + |
| 177 | Carbohydrate | + | - |
| | Steroid | ++ | + |
| | Resin | ++ | ++ |

178 **Key:** + Low concentration,
179 ++ Medium concentration
180 +++ High concentration
181 - No activity

182 **Antimicrobial Activity of Extracts.**

183 **The Preliminary Tests**

184 The Methanol extract had great spectrum of activity against the test organisms at different concentration. However,
185 *Salmonella typhi* showed no activity in the two plants extracts respectively. Clinical isolate of *Bacillus subtilis* and
186 *Escherichia coli* were most susceptible as they displayed the widest zones of inhibition against the test organisms,
187 using the two plant extracts respectively (Table 3 and 4). *Staphylococcus aureus* and *Pseudomonas aeruginosa*
188 showed moderate zones of inhibition at different concentration in *Bryocarpus coccineus* when compared with
189 *Zanthoxylum piperitum* extract, where *Staphylococcus aureus* showed zones of inhibition on concentration of
190 100mg/ml (10mm), 50mg/ml (10mm) and 25mg/ml (9mm) respectively. *Pseudomonas aeruginosa* showed
191 activities in higher concentrations except at 6.25mg/ml and 3.125mg/ml respectively. *Klebsiella pneumonia* showed

192 no zones of inhibition with *Bryocarpus coccineus* extract, while *Zanthoxylum piperitum* extract inhibited it at
193 different concentration.

194 Antimicrobial Activity of the Extracts on the Type Culture

195 *B. coccineus* extract had antimicrobial activity on *S. aureus* (15mm) and *E. coli* (16mm), while *Z. piperitum*
196 recorded activity on against *S. aureus* (13mm) and *E. coli* (10mm). Both extracts had no activity on *P. aeruginosa*.

197 Comparison of the Two Extract on Test Isolates

198 The two plants extract were checked for their activity on the test organisms. The extract with the highest activity on
199 a particular plant extract was determined. In Fig.3, 4 and Fig 5, it was found that *B. coccineus* had the highest
200 activity IZD (14mm, 12mm and 13mm). In Fig. 7, where they were tested against *K. Pneumonia*, *Z. piperitum* had
201 the highest activity IZD (12mm).

202 Comparison of the Antibacterial Activity of the Leaf Extracts of *B. Coccineus*, *Z. Piperitum* and 203 Ciprofloxacin.

204 The two extracts and ciprofloxacin were tested against the Test organisms and it was observed that Ciprofloxacin
205 had the highest IZD (35µg/ml) at the concentration of 100mg/ml against *B. subtilis* (Table 6).

206 **TABLE 3: Antibacterial Activity of Extracts of *Bryocarpus coccineus*.**

207

208

| Organism/conc | Inhibitory Zone Diameter (IZD) In mm | | | | | |
|----------------------|--------------------------------------|---------|---------|-----------|-----------|------------|
| | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.25mg/ml | 3.125mg/ml |
| <i>B. subtilis</i> | 14 | 13 | 12 | 11 | 10 | 8 |
| <i>Staph. aureus</i> | 12 | 12 | 9 | 9 | 8 | 8 |
| <i>E. coli</i> | 13 | 12.4 | 11.6 | 11 | 10 | 9 |
| <i>P. aeruginosa</i> | 11 | 10 | 9.6 | 9 | 8 | 7 |
| <i>K. pneumonia</i> | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>S. typhii</i> | 0 | 0 | 0 | 0 | 0 | 0 |

209

TABLE 4: Antibacterial Activity of Extracts of *Zanthoxylum piperitum*

| Organism/Conc | Inhibitory Zone Diameter (IZD) (mm) | | | | | |
|----------------------|-------------------------------------|---------|---------|-----------|-----------|------------|
| | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.25mg/ml | 3.125mg/ml |
| <i>B. subtilis</i> | 11 | 9 | 9 | 8.5 | 8 | 7 |
| <i>S. aureus</i> | 10 | 10 | 9.5 | 9 | 0 | 0 |
| <i>E. coli</i> | 11 | 10 | 9.5 | 9.2 | 8.9 | 8.4 |
| <i>P. aeruginosa</i> | 11 | 10 | 9 | 0 | 0 | 0 |
| <i>K. pneumonia</i> | 12 | 11 | 10 | 9.2 | 8 | 7 |
| <i>S. typhi</i> | 0 | 0 | 0 | 0 | 0 | 0 |

210

211 **TABLE 5: Antimicrobial activity of the extract on the Type culture**

| Test organism | <i>B. coccineus</i> | <i>Z. piperitum</i> |
|----------------------|---------------------|---------------------|
| <i>S. aureus</i> | 15 | 13 |
| <i>E. coli</i> | 16 | 10 |
| <i>P. aeruginosa</i> | - | - |

212

Key: - no activity

213 **TABLE 6: Comparism of Antibacterial activity of the leaf extracts of *B. coccineus*, *Z. piperitum* and**
214 **Ciprofloxacin.**

215

| Bacterial isolates | Mean Inhibition Zone Diameter (mm) | | |
|---|------------------------------------|---------------------|-------------------------|
| | <i>B. coccineus</i> | <i>Z. piperitum</i> | Ciprofloxacin (25µg/ml) |
| <i>E. coli</i> (clinical isolate) | 11.17 | 9.50 | 30.00 |
| <i>E. coli</i> ATCC 2127 | 16.00 | 10.00 | 31.5 |
| <i>Staph. aureus</i> (clinical isolate) | 9.67 | 9.63 | 27.00 |
| <i>S. aureus</i> ATCC 2278 | 15.00 | 13.00 | 24.00 |
| <i>P. aeruginosa</i> (clinical isolate) | 9.10 | 10.00 | 28.5 |
| <i>P. aeruginosa</i> ATCC 2078 | 0.00 | 0.00 | 26.00 |
| <i>B. subtilis</i> (clinical isolate) | 11.33 | 8.75 | 35.00 |
| <i>K. pneumoniae</i> (clinical isolate) | 0.00 | 9.53 | 19.00 |
| <i>S. typhi</i> (clinical isolate) | 0.00 | 0.00 | 28.00 |

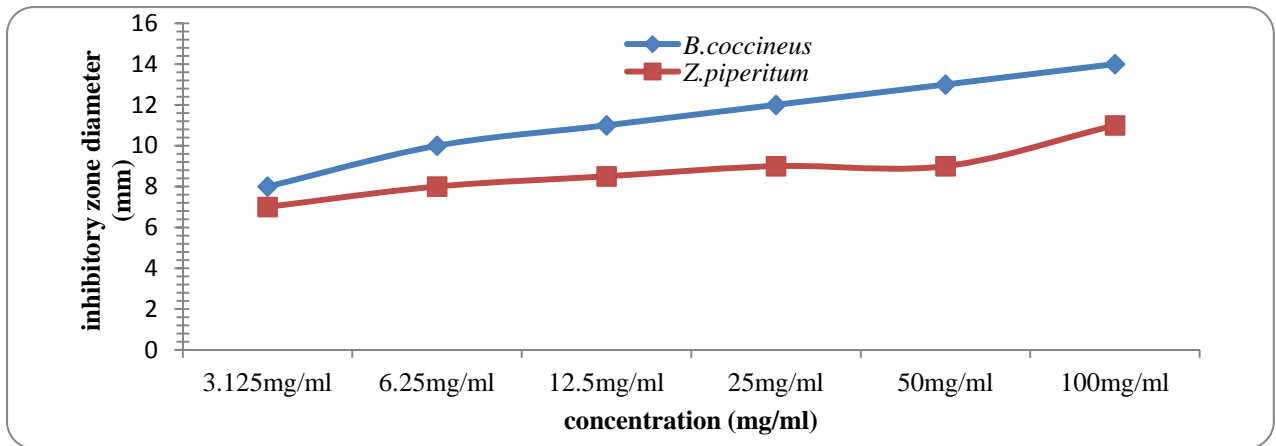


Fig.3: Antibacterial activity of the two plants extract on *B. subtilis*

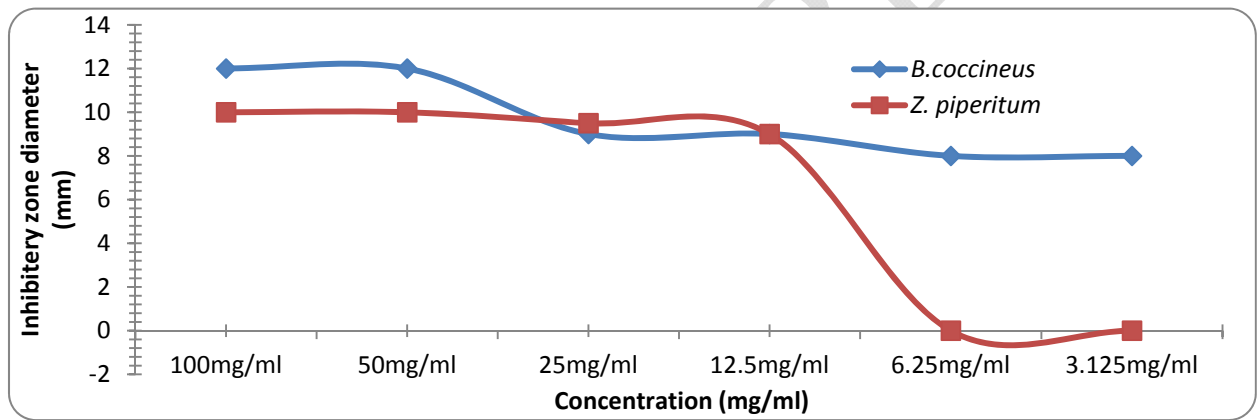


Fig.4: Antibacterial activity of the two plants extract on *S. aureus*.

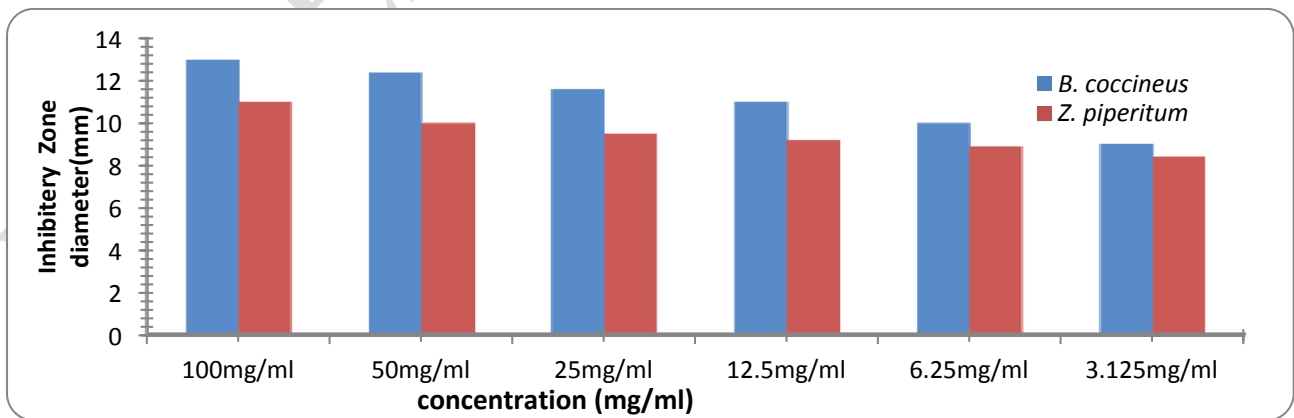
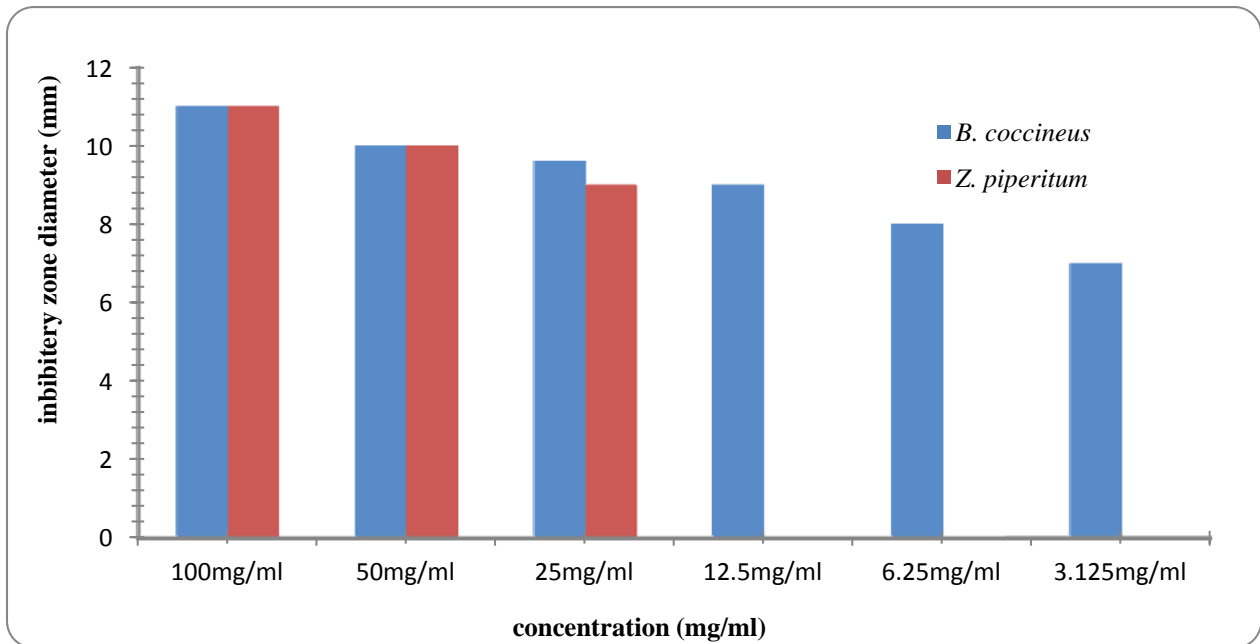


Fig. 5 Antibacterial activity of the two plants extract on *Escherichia coli*.



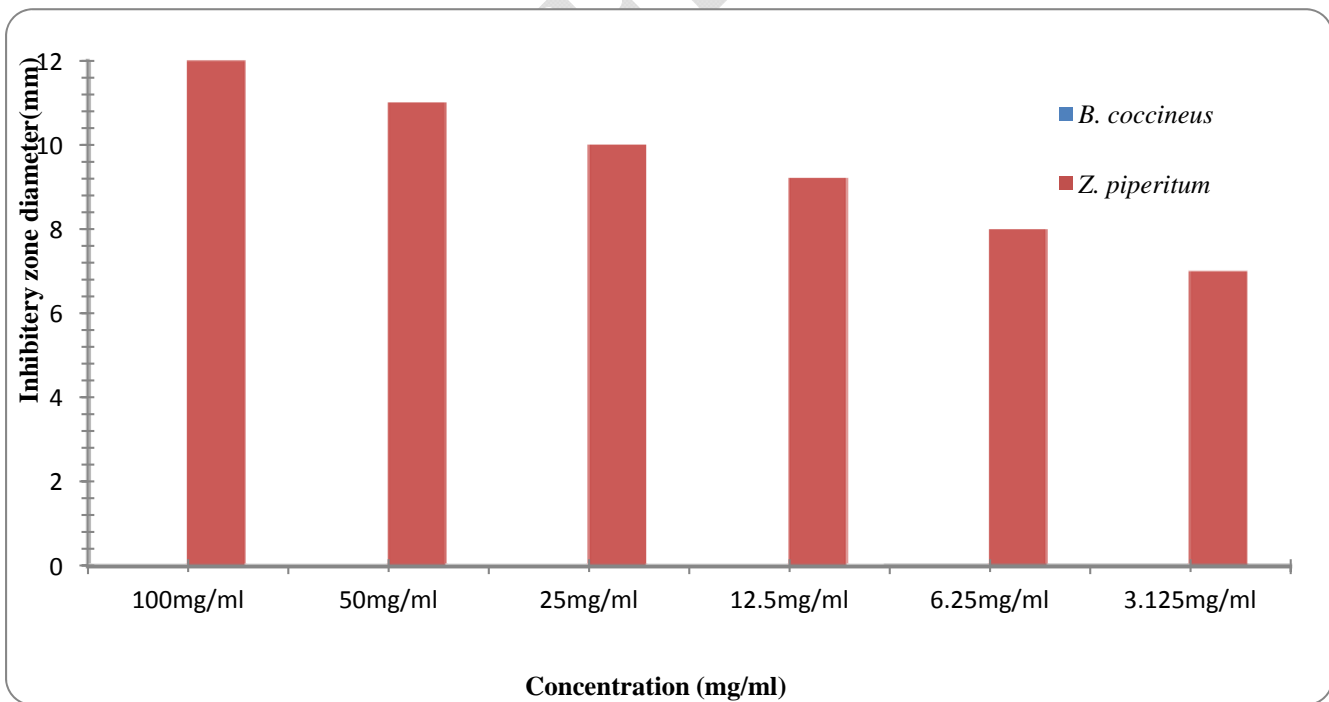
223

224

225

226

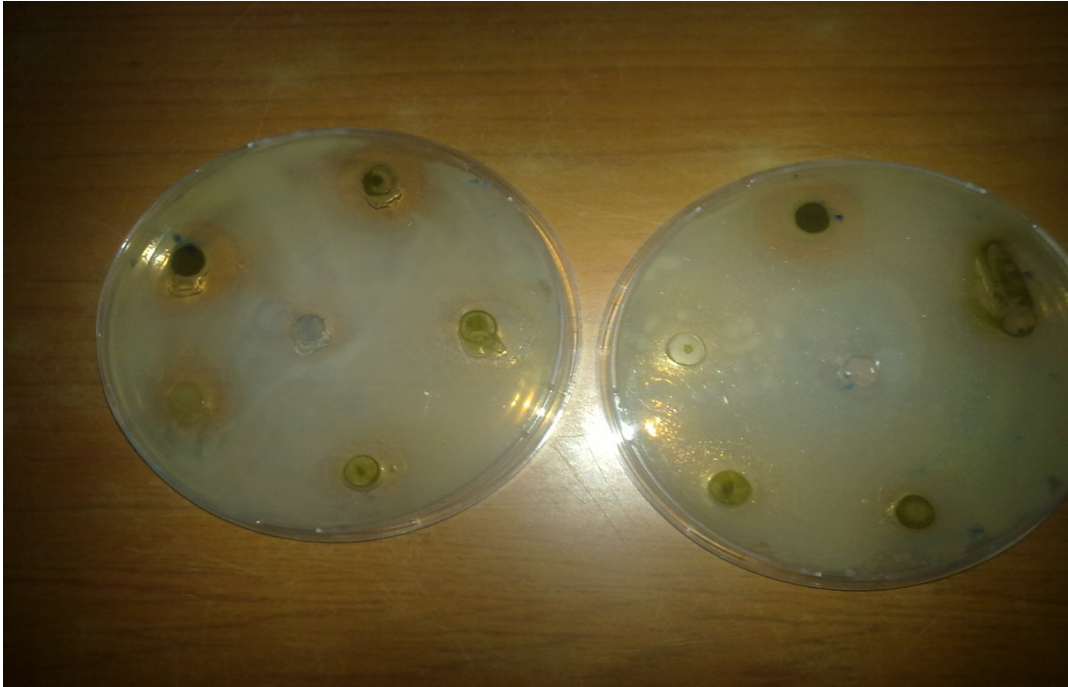
Fig.6: Antibacterial activity of the two plants extract on *Pseudomonas aeruginosa*.



227

228

Fig.7: Antibacterial activity of the two plants extract on *Klebsiella pneumoniae*.



230

231 **Plate 1: Inhibition zone diameter of *Brysocarpus coccineus* and *Zanthoxylum piperitum* methanol extract on**
 232 **microorganisms.**

233

234

TABLE 7: Results of MIC for Methanolic Extract of *Brysocarpus coccineus* leaves

| Test organisms | Concentrations (mg/ml) | | | | | |
|----------------------|------------------------|----|----|------|------|-------|
| | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 |
| <i>S. aureus</i> | - | - | - | - | + | + |
| <i>K. pneumoniae</i> | + | + | + | + | + | + |
| <i>B. subtilis</i> | - | - | - | - | - | + |
| <i>S. typhi</i> | + | + | + | + | + | + |
| <i>P. aeruginosa</i> | - | - | - | - | + | + |
| <i>E. coli</i> | - | - | - | - | - | + |

235

Key: - = No growth

236

+ = Growth

237

238 **TABLE 8: Results of MIC for Methanol Extract of *Zanthoxylum piperitum* Leaves**

| Test organisms | Concentrations (mg/ml) | | | | | |
|----------------------|------------------------|----|----|------|------|-------|
| | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 |
| <i>S. aureus</i> | - | - | - | - | + | + |
| <i>K. pneumonia</i> | - | - | - | + | + | + |
| <i>B. subtilis</i> | - | - | - | - | - | + |
| <i>S. typhi</i> | + | + | + | + | + | + |
| <i>P. aeruginosa</i> | - | - | - | + | + | + |
| <i>E. coli</i> | - | - | - | + | + | + |

239 Key: - = No growth
 240 + = Growth

241
 242 **Table 8: Interpretation the MIC results**
 243

| Test organisms | Minimum Inhibitory Diameter (mg/mL) | |
|----------------------|-------------------------------------|---------------------|
| | <i>B. coccineus</i> | <i>Z. piperitum</i> |
| <i>S. aureus</i> | 12.5 | 6.25 |
| <i>K. pneumonia</i> | 0 | 25 |
| <i>B. subtilis</i> | 6.25 | 6.25 |
| <i>S. typhi</i> | 0 | 0 |
| <i>P. aeruginosa</i> | 12.5 | 25 |
| <i>E. coli</i> | 6.25 | 25 |

244
 245 **Result of the Statistical Analysis for *B. coccineus* Extract**

246 At 95% Significant Level, F tabulated = 3.29, F calculated = 3.6482 . Since, $F_{cal} > F_{tab}$; this shows that the
 247 treatment means was not equal i.e. there was significant difference between the treatment means. The
 248 least significant difference (LSD) value between the means is 3.0009. However, in ranking of the means,
 249 the difference between the consecutive means is < LSD value. This shows that there was no significant
 250 difference between the means.

251 **TABLE 9: ANOVA for the activities of *B. coccineus* on the Test organisms.**

| Source of Variation | sums of squares | d.f | means of squares | Fcal |
|---------------------|-----------------|-----|------------------|--------|
| Treatment | 21.9543 | 3 | 7.3181 | 3.6482 |
| Error | 40.119 | 20 | 2.0060 | |
| Total | 62.0733 | 23 | | |

252

253 **Result of the Statistical Analysis for *Z. piperitum* Extract**

254 At 95% confidence interval, F tabulated was 2.25 and F calculated was 6024. Since, $F_{cal} < F_{tab}$; this shows
 255 that the treatment means were not equal hence there was significant difference between the treatment
 256 means. The least significant difference (LSD) value between the means equals 5.5287. However, in
 257 ranking of the means, the difference between the consecutive means was $>LSD$ value. This means that
 258 there was a significant difference between the means.

259 **TABLE 10: ANOVA for the activities of *Z. piperitum* on the Test organisms.**

| Sources of Variance | Sums of Square | D.F | Means of Square | Fcal. |
|---------------------|----------------|-----|-----------------|--------|
| Treatment | 3.9826 | 20 | 0.9957 | 0.6024 |
| Error | 33.0558 | 20 | 1.6528 | |
| Total | 37.0384 | 24 | | |

260

261 **4.0 DISCUSSION**

262 The methanol extracts of *Bryocarpus coccineus* and *Zanthoxylum piperitum* leaves were evaluated for
 263 their antimicrobial activity against the following organisms: Clinical isolates of *E.coli*, *S. aureus*, *B.*
 264 *subtilis*, *P. aeruginosa*, *K. pneumonia*, *S. typhi* and typed strain of *S. aureus* (ATCC 2278), *E .coli* (ATCC
 265 2127), and *P. aeruginosa* (UCH 2078) . The percentage yield determined showed 5.6% for *Bryocarpus*
 266 *coccineus* and 4% for *Zanthoxylum piperitum* for methanol extractions. This revealed that methanol
 267 extract more yield of *Bryocarpus coccineus* than with *Zanthoxylum piperitum* leaves. Since the

268 composition of methanol is the same, a plausible explanation for the greater extraction power of methanol
269 in *B. coccineus* over *Z. piperitum* may be as a result of the composition of the plant material and as a
270 result of methanol to extract both polar and non-polar compounds that constitute the plant's structure.

271 Plant extracts has been reported to have antimicrobial activity against various microorganisms [16]. In
272 this study, it was found that the methanol extract exhibited a lot of antibacterial activity. The result of
273 phytochemical analysis using the method described by Harborne [15], revealed that the methanol extract
274 of *B. coccineus* contained Saponins in high concentration (+++), glycosides, flavonoids, resins and steroid
275 in moderate concentration (++), and Carbohydrates in low concentration (+). This can be attributable to
276 the presence of alkaloid in the extract. Alkaloid has been reported to have antimicrobial activity against a
277 variety of microorganism including bacteria [17]. *Z. piperitum* contained alkaloid, tannins, glycosides and
278 resins in moderate concentration (++), while saponins, flavonoids and steroids are in low concentration
279 (+) as indicated in Table 2. The presence of saponins and Tannin in these extracts is believed to contribute
280 to the enhanced antimicrobial activity of the extracts. This is in line with the fact that they are
281 antimicrobial agents found in plant materials [18]. The secondary metabolites of plant are the bioactive
282 constituents of plant extracts [19]

283 The methanol extract of *B. coccineus* was active against some of the Clinical isolate; *B. subtilis*, *S.*
284 *aureus*, *E. coli* and *P. aeruginosa* at different concentration, while that of *Z. piperitum* showed activity
285 against *B. subtilis*, *E.coli*, *K. pneumonia* at different concentration. *Z. piperitum* extract had activity on *S.*
286 *aureus* at 100mg/ml to 12.5mg/ml concentration, and on *P. aeruginosa* at 100mg/ml to 25mg/ml
287 concentration. The higher activity of the methanol extract is attributable to the presence of flavonoid
288 compound (Table 2). Our findings in this study are in agreement with the report that flavonoid
289 compounds are active against bacteria pathogens [20]. The presence of saponins and Tannin in these
290 extracts is believed to contribute to the enhanced antimicrobial activity of the extracts. This is in line with
291 the fact that there are antimicrobial agents found in the plant material [18]

292 The results of the minimum inhibitory concentration (MIC) of the *B. coccineus* showed that the
293 methanolic extract had the least inhibitory concentration (6.25mg/ml) against Clinical isolate of *E. coli*
294 and *B. subtilis*. The MIC of *Z. piperitum* methanolic extract was 6.25 mg/ml against clinical isolate *B.*
295 *subtilis*. All the plants extract had no activity against *S. typhi*, and *B. coccineus* had no activity against *K.*
296 *pneumonia*. The results of the minimum inhibitory concentration revealed the importance of leaf extracts
297 of *B. coccineus* and *Z. piperitum* in control resistant strains which are becoming a threat to human health.

298 The results of statistical analysis (ANOVA) of the *B. coccineus* showed that F-cal. is greater than F-tab.
299 This means that there is a significant difference on the activity of the extract. Statistical analysis of *Z.*
300 *piperitum* showed that F-cal is less than F-tab. meaning there is no significant difference on the activity of
301 the extract.

302 **5.0 CONCLUSION**

303 Some of the claims by native herbal practitioners on the medicinal values of *Brysocarpus coccineus* and
304 *Zanthoxylum piperitum* have been validated by this work. Chemotherapy as a common denominator for
305 the modern medicine and pharmacy actually started from plants medicine. Antimicrobial chemicals can
306 therefore be isolated from medicinal plants found within our locality and this has proved to be as effective
307 as the antibiotics in use. It is really amazing the health benefits of the leaves of these plants; ranging from
308 the antimicrobial action, to pain relieving effect, spasm reduction, depression of CNS, lowering blood
309 pressure, reduction of blood sugar, as well as promotion of fertility and including many others. The
310 leaves of these plant are found everywhere in our neighborhood, garden, farmland, jungle or forest. This
311 is a gift of nature to mankind. They should not just be allowed to waste away in the jungle, but its
312 potentials has to be harnessed, and especially in an era where greater numbers of antibiotics are getting
313 increasingly inactive due to the ever-mutating and resistant microorganisms.

314

315 **AUTHORS' CONTRIBUTIONS**

316 This work was carried out in collaboration among all authors

317 **CONSENT**

318 As per international standard or university standard written participant consent has been collected and
319 preserved by the authors.

320 **COMPETING INTERESTS**

321 Authors have declared that no competing interests exist.

322 **REFERENCES**

- 323 1. Adebayo EA, Ishola OR, Majolagbe ON. Evaluation of the Methanol extracts of *Ficus exasperata*
324 stems, leaf and root for Phytochemistry and Antimicrobial Activities. African Journal of Plant
325 Science. 2009; 3(12): 283-287.
- 326 2. Wazis CH, Anuka JA, Timothy SY, Zezi AU, Mohammed GT, Hussaini IM. Acute Toxicity and in-
327 vivo effects of leaf extracts of *Brysocarpus Coccineus* Shum &Thonn in Pregnant Rat Uterus.
328 Journal of Applied Pharmaceutical Science. 2012; 2 (12): 130-136
- 329 3. Gill LS. Ethnomedical Uses of Plants in Nigeria. Uniben Press, Benin City. 2000; 15-65.
- 330 4. Ejeh SA, Onyeyili P, Abalaka SE. Anti-diarrhea activity of the aqueous root bark extract of
331 *Byrsocarpus coccineus* on castor oil-induced diarrhea in Wistar rats, Veterinary World. 2017; 10(7):
332 743-747.
- 333 5. Chorpaka P, Nuchnipa N, Hataichanoke N. Antifungal Activity of the Essential Oil Extracted from
334 *Zanthoxylum piperitum* Seeds Against *Aspergillus flavus* . Chiang Mai J. Sci. 2017; 44(2): 584-594.

- 335 6. Walla FM, Bushra HS, Reem NI and Mohammed BH. Antibacterial Activity of *Zingiber officinale*
336 (Ginger) against Clinical Bacterial Isolates. South Asian Journal of Research in Microbiology.2019;
337 3(2): 1-7.
- 338 7. Essien AD, Essiet GA, Akuodor GC, Akpan JL, Chilaka KC, Bassey AL, Ezeokpo BC and
339 Nwobodo NN. Pharmacological evaluation of the aqueous stem barks extract of *Bombax*
340 *buonopozense* in the relief of pain and fever. African Journal of Pharmacy and Pharmacology 2016;
341 10(5): 59-65.
- 342 8. Akindele AJ and Adeyemi OO. Anxiolytic and sedative effects of *Bryocarpus coccineus* Schum and
343 Thonn. (Connaraceae) extract. International Journal of Applied Research in Natural Plant. 2007; 3(1):
344 28-36.
- 345 9. Oke JM, Hamburger MO. Screening of some Nigerian medicinal plant for antioxidant activity using
346 2-2-Diphenyl-picryl-hydrazyl radical. African Journal of Biomedical Research. 2002; 5(1-2): 77-79.
- 347 10. Akindele AJ, and Adeyemi OO. Anxiolytic and sedative effects of *Bryocarpus coccineus* Schum and
348 Thonn. (Connaraceae) extract. International Journal of Applied Research in Natural Plant 2010; 3(1):
349 28-36.
- 350 11. Akindele AJ, Adeyemi OO. Analgesic activities of the aqueous leaf extract of *Bryocarpus coccineus*.
351 Nigerian Journal of Health and Biomedical Science. 2006; 5(1): 43-46.
- 352 12. Ahamadu AA, Hassan HS, Abubakar MU. Flavonoids glucoside from *Bryocarpus coccineus* leaves
353 Schum and Thonn Connaraceae). Africa Journal of Traditional, Complimentary and Alternative
354 Medicines. 2007; 4(3): 257-260.
- 355 13. Yamazaki E, Inagaki M, Kurita O, Inoue T. Antioxidant activity of Japanese pepper (*Zanthoxylum*
356 *piperitum* DC.) fruit. Food Chemistry. 2007; 100:171–177.
- 357 14. Chorpaka P, Nuchinpa N., and Hataichanoke N. Antifungal Activities of the essential oil extracted
358 from *Zanthoxylum piperitum* seeds against *Aspergillus Flavus*. Chiang Mai Journal of Science 2017;
359 44(2): 584-594.

- 360 15. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd
361 Edition. Chapman and Hall, London, ISBN: 0-412-57270-2. 1998; 302.
- 362 16. Gislene G, Locatelli J, Freitas P, and Silva G. Antibacterial Activity of Plant Extracts and
363 Phytochemicals on Antibiotic-Resistant Bacteria. *Brazilian Journal of Microbiology*. 2000; 31(4):
364 1517-1590.
- 365 17. Jain S, and Sherma R. Antimicrobial Activity of Pyrrolizidine Alkaloids from *Heliotropium*
366 *ellipticum*. *Journal of Chemistry and Pharmacology*. 1986; 35 (8): 3487-3489.
- 367 18. Ajali U. *Chemistry of Biocompounds*. 1st Edition, Rhyce Kerex Publishers, Enugu. 2004; pp 97 and
368 118.
- 369 19. Bobbarala V, Katikala P, Naidu K. and Penumajji S. Antifungal Activity of Selected Plant Extracts
370 against pathogenic Fungi *Aspergillus niger* F2723. *Indian Journal of Science and Technology*. 2009;
371 2 (4): 0974-6846
- 372 20. Seo YY, Sang-Hyun L, Bui HT, Hae-Dong J, and Young HK. Antioxidant and Anti-Osteoporosis
373 Activities of Chemical Constituents of the Stems of *Zanthoxylum piperitum*. *Molecules* 2018; 23-
374 457.

375
376