

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 *Bryocarpus*
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 *Bryocarpus 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 *Bryocarpus 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 *Bryocarpus 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 *Bryocarpus coccineus*. The
49 leaves were obtained from different locations in Nsukka; Obukpa (6° 54' 0" North, 7° 24' 0" East), Ehandiagu-Eha-

50 Alumona (6° 50' 0" North, 7° 27' 0" East) and Orba (Latitude: 6°51'14.76", Longitude: 7°27'45") during the dry
51 season. *Bryocarpus coccineus* was got from an open farm-land in Ehandiagu-Eha- Alumona (6° 50' 0" North, 7°
52 27' 0" East), and *Zanthoxylum piperitum* was got from a thick forest in Orba area ((Latitude: 6°51'14.76",
53 Longitude: 7°27'45"). The leaves were identified by Mr Ozioko from Bioresource Development and Conservation
54 Programme, Nsukka. These plants were then air-dried in the lab at room temperature for about 5 days. The dried
55 parts were subsequently reduced to fine powder using an electric waring blender (model 51BL30)

56 2.2. Methanol extraction of anti-microbial substances from *Bryocarpus coccineus* and *Zanthoxylum* 57 *piperitum* leaves.

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

63 2.3. Percentage Yield of Extract

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

$$65 \text{ Percentage yield (\% yield)} = \frac{\text{mass of dried extract (g)}}{\text{Mass of pulverized plant material (g)}} \times 100 \dots\dots (1)$$

66 2.4. Phytochemical Analysis

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

71 2.4.1. Test for Alkaloids

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

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

76 **2.4.2. Test for Tannins:**

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

80 **2.4.3. Test for Flavonoids**

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

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

87 **2.4.4. Test for Saponins**

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

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

94 **2.4.5. Test for Glycosides**

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

- 97 • 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
98 alkaline (tested with litmus paper). This was heated on a water bath for 2 minutes. The precipitate obtained
99 was observed for brick red coloration.
- 100 • Using 15 ml of dilute sulphuric acid instead of water, the above process was repeated and the quantity of
101 precipitate formed was observed and compared with that of the former experiment.

102 **2.4.6. Test for Steroids**

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

105 **2.4.7. Test for Terpenoids**

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

108 **2.4.8. Test for Carbohydrates**

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

113 **2.4.9. Test for Resins**

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

116

117 **2.5. SCREENING FOR ANTIMICROBIAL ACTIVITY**

118 **2.5.1. Test Microorganisms**

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

125 **2.5.2. Preparation of 0.5 McFarland Standard**

126 Briefly, 1.175g of Barium chloride crystal (BaCl_2) were weighed out and dissolved in 100ml distilled water to give
127 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
128 to 99ml of distilled water to make 1% H_2SO_4 solution. Furthermore, 9.95ml of 1% H_2SO_4 solution was mixed with
129 0.05ml of 1.175% Barium chloride solution to make 0.5 McFarland's standard. This was used to standardize the test
130 isolates.

131 **2.5.3. Preliminary Screening for the Antibacterial Activities of the extracts using the Agar Diffusion** 132 **Technique.**

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

141 **2.5.4. Determination of the Minimum Inhibitory Concentration (MIC) using the Broth Micro dilution** 142 **Technique**

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

148

149 2.6. STATISTICAL ANALYSIS

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

153 3.0. RESULTS

154 Percentage Yield

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

157

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

159

160 Methanol extracts	160 % Yields
161 <i>B. coccineus</i>	161 5.6
162 <i>Z. piperitum</i>	162 4.0

163

164

165

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166 Phytochemical Analysis

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

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

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

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

183 **Antimicrobial Activity of Extracts.**

184 **The Preliminary Tests**

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

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

195 Antimicrobial Activity of the Extracts on the Type Culture

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

198 Comparison of the Two Extract on Test Isolates

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

203 Comparison of the Antibacterial Activity of the Leaf Extracts of *B. Coccineus*, *Z. Piperitum* and 204 Ciprofloxacin.

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

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

208

209

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

210

211

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

212

213

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>	-	-

214

Key: - no activity

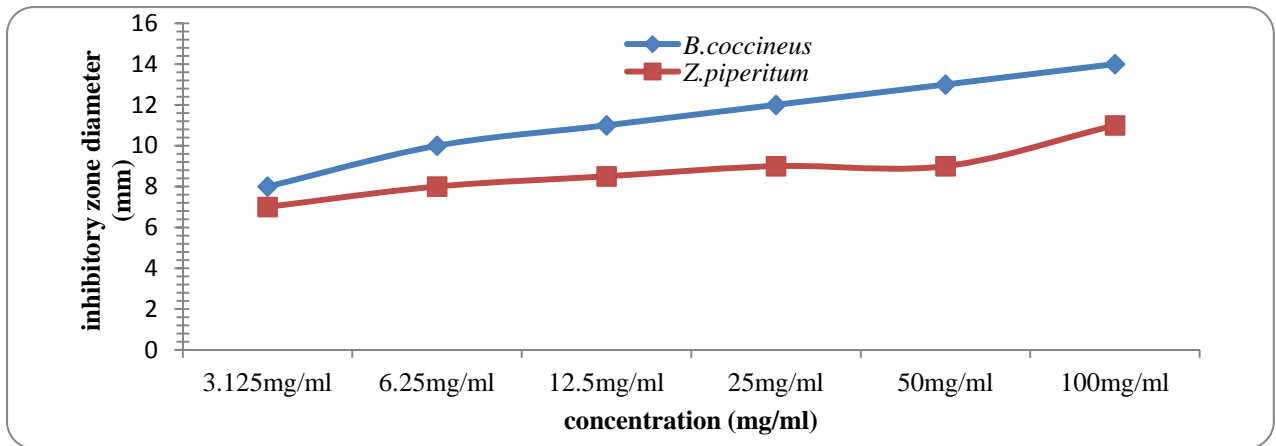
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TABLE 6: Comparism of Antibacterial activity of the leaf extracts of *B. coccineus*, *Z. piperitum* and Ciprofloxacin.

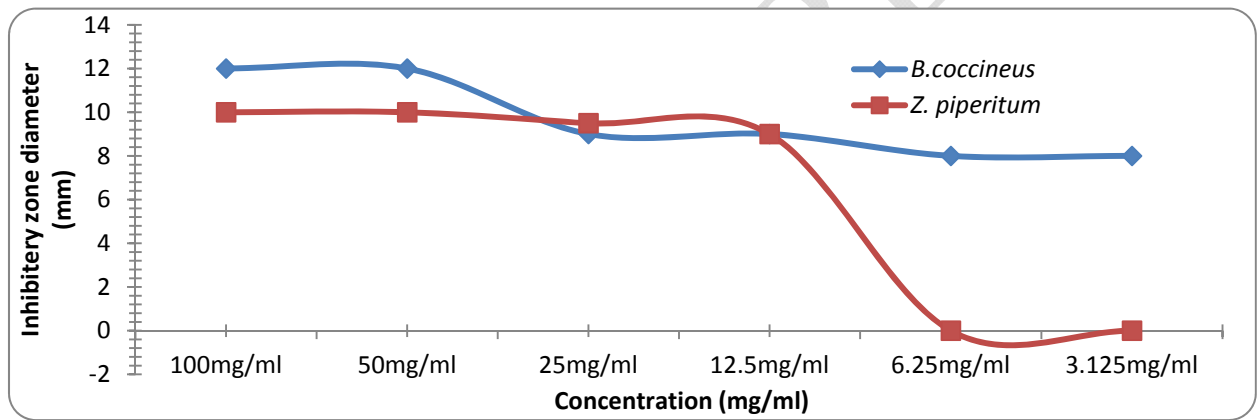
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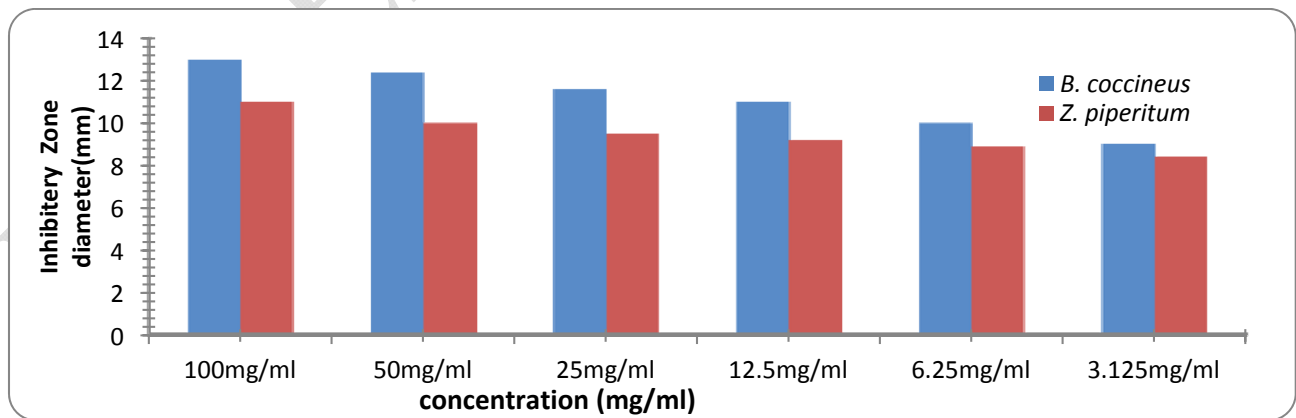
Bacterial isolates	Mean Inhibition Zone Diameter (mm)		
	<i>B. coccineus</i>	<i>Z. piperitum</i>	Ciprofloxacin (25µg/ml)
<i>E. coli</i> (clinical isolate)	11.17	9.50	30.00
<i>E. coli</i> ATCC 2127	16.00	10.00	31.5
<i>Staph. aureus</i> (clinical isolate)	9.67	9.63	27.00
<i>S. aureus</i> ATCC 2278	15.00	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



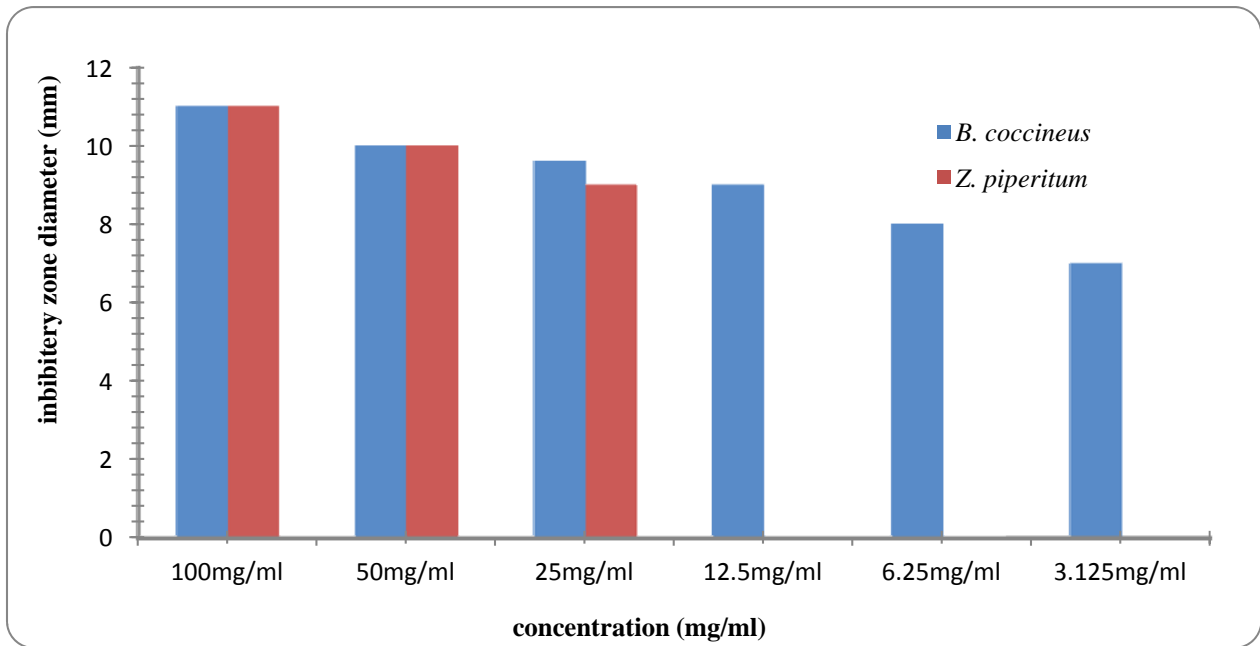
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219 **Fig.3: Antibacterial activity of the two plants extract on *B. subtilis***
220



221
222 **Fig.4: Antibacterial activity of the two plants extract on *S. aureus*.**



223
224 **Fig. 5 Antibacterial activity of the two plants extract on *Escherichia coli*.**



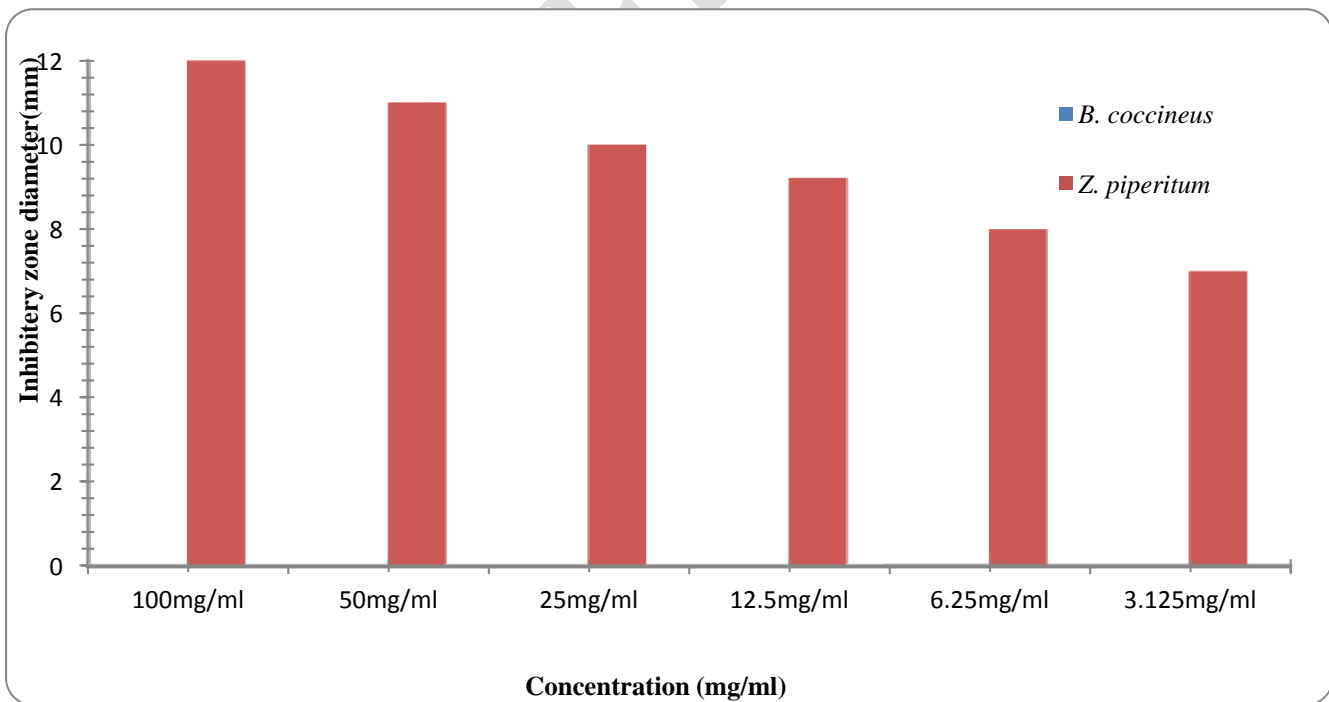
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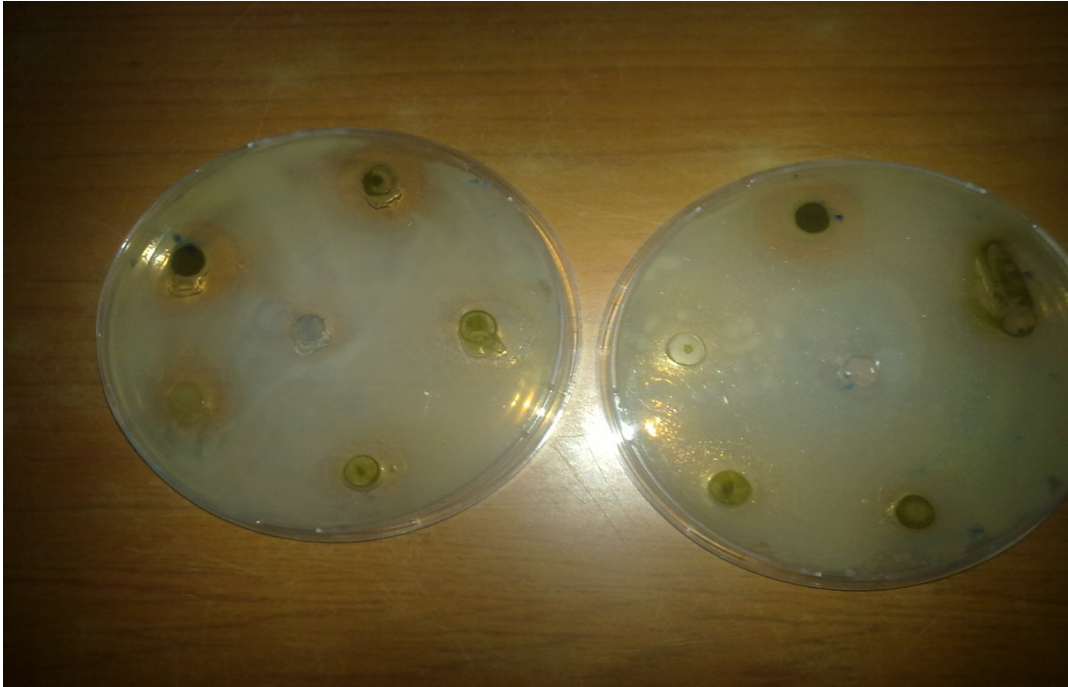
Fig.6: Antibacterial activity of the two plants extract on *Pseudomonas aeruginosa*.



229

230

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



232

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

235

236

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>	-	-	-	-	-	+

237

Key: - = No growth

238

+ = Growth

240 **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>	-	-	-	+	+	+

241 Key: - = No growth
 242 + = Growth

243
 244 **Table 8: Interpretation the MIC results**
 245

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

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

253 **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		

254

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

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

261 **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		

262

263 **4.0 DISCUSSION**

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

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

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

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

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

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

304 5.0 CONCLUSION

305 The present study has demonstrated that *Brysocarpus coccineus* and *Zanthoxylum piperitum* has
306 antimicrobial activity against clinical isolates of *E.coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K.*
307 *pneumonia* and *S. typhi*. So many studies have been carried out on the medicinal values of *Brysocarpus*
308 *coccineus* and *Zanthoxylum piperitum* extracts leading to the isolation of highly medicinal
309 phytochemicals, thus validating some of its documented traditional use in the treatment of illness across
310 Africa. These extracts show great promise especially in the advert of multidrug resistant strains of such
311 isolates.

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314 AUTHORS' CONTRIBUTIONS

315 This work was carried out in collaboration among all authors

316 **CONSENT**

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

319 **COMPETING INTERESTS**

320 Authors have declared that no competing interests exist.

321 **REFERENCES**

- 322 1. Adebayo EA, Ishola OR, Majolagbe ON. Evaluation of the Methanol extracts of *Ficus exasperata*
323 stems, leaf and root for Phytochemistry and Antimicrobial Activities. African Journal of Plant
324 Science. 2009; 3(12): 283-287.
- 325 2. Wazis CH, Anuka JA, Timothy SY, Zezi AU, Mohammed GT, Hussaini IM. Acute Toxicity and in-
326 vivo effects of leaf extracts of *Brysocarpus Coccineus* Shum &Thonn in Pregnant Rat Uterus.
327 Journal of Applied Pharmaceutical Science. 2012; 2 (12): 130-136
- 328 3. Gill LS. Ethnomedical Uses of Plants in Nigeria. Uniben Press, Benin City. 2000; 15-65.
- 329 4. Ejeh SA, Onyeyili P, Abalaka SE. Anti-diarrhea activity of the aqueous root bark extract of
330 *Byrsocarpus coccineus* on castor oil-induced diarrhea in Wistar rats, Veterinary World. 2017; 10(7):
331 743-747.
- 332 5. Chorpaka P, Nuchnipa N, Hataichanoke N. Antifungal Activity of the Essential Oil Extracted from
333 *Zanthoxylum piperitum* Seeds Against *Aspergillus flavus* . Chiang Mai J. Sci. 2017; 44(2): 584-594.
- 334 6. Walla FM, Bushra HS, Reem NI and Mohammed BH. Antibacterial Activity of *Zingiber officinale*
335 (Ginger) against Clinical Bacterial Isolates. South Asian Journal of Research in Microbiology.2019;
336 3(2): 1-7.
- 337 7. Essien AD, Essiet GA, Akuodor GC, Akpan JL, Chilaka KC, Bassey AL, Ezeokpo BC and
338 Nwobodo NN. Pharmacological evaluation of the aqueous stem barks extract of *Bombax*

- 339 *buonopozense* in the relief of pain and fever. African Journal of Pharmacy and Pharmacology 2016;
340 10(5): 59-65.
- 341 8. Akindele AJ and Adeyemi OO. Anxiolytic and sedative effects of *Bryocarpus coccineus* Schum and
342 Thonn. (Connaraceae) extract. International Journal of Applied Research in Natural Plant. 2007; 3(1):
343 28-36.
- 344 9. Oke JM, Hamburger MO. Screening of some Nigerian medicinal plant for antioxidant activity using
345 2-2-Diphenyl-picryl-hydrazyl radical. African Journal of Biomedical Research. 2002; 5(1-2): 77-79.
- 346 10. Akindele AJ, and Adeyemi OO. Anxiolytic and sedative effects of *Bryocarpus coccineus* Schum and
347 Thonn. (Connaraceae) extract. International Journal of Applied Research in Natural Plant 2010; 3(1):
348 28-36.
- 349 11. Akindele AJ, Adeyemi OO. Analgesic activities of the aqueous leaf extract of *Bryocarpus coccineus*.
350 Nigerian Journal of Health and Biomedical Science. 2006; 5(1): 43-46.
- 351 12. Ahamadu AA, Hassan HS, Abubakar MU. Flavonoids glucoside from *Bryocarpus coccineus* leaves
352 Schum and Thonn Connaraceae). Africa Journal of Traditional, Complimentary and Alternative
353 Medicines. 2007; 4(3): 257-260.
- 354 13. Yamazaki E, Inagaki M, Kurita O, Inoue T. Antioxidant activity of Japanese pepper (*Zanthoxylum*
355 *piperitum* DC.) fruit. Food Chemistry. 2007; 100:171–177.
- 356 14. Chorpaka P, Nuchinpa N., and Hataichanoke N. Antifungal Activities of the essential oil extracted
357 from *Zanthoxylum piperitum* seeds against *Aspergillus Flavus*. Chiang Mai Journal of Science 2017;
358 44(2): 584-594.
- 359 15. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd
360 Edition. Chapman and Hall, London, ISBN: 0-412-57270-2. 1998; 302.
- 361 16. Gislene G, Locatelli J, Freitas P, and Silva G. Antibacterial Activity of Plant Extracts and
362 Phytochemicals on Antibiotic-Resistant Bacteria. Brazilian Journal of Microbiology. 2000; 31(4):
363 1517-1590.

- 364 17. Jain S, and Sherma R. Antimicrobial Activity of Pyrrolizidine Alkaloids from *Heliotropium*
365 *ellipticum*. Journal of Chemistry and Pharmacology. 1986; 35 (8): 3487-3489.
- 366 18. Ajali U. Chemistry of Biocompounds. 1st Edition, Rhyce Kerex Publishers, Enugu. 2004; pp 97 and
367 118.
- 368 19. Bobbarala V, Katikala P, Naidu K. and Penumajji S. Antifungal Activity of Selected Plant Extracts
369 against pathogenic Fungi *Aspergillus niger* F2723. Indian Journal of Science and Technology. 2009;
370 2 (4): 0974-6846
- 371 20. Seo YY, Sang-Hyun L, Bui HT, Hae-Dong J, and Young HK. Antioxidant and Anti-Osteoporosis
372 Activities of Chemical Constituents of the Stems of *Zanthoxylum piperitum*. Molecules 2018; 23-
373 457.
- 374
- 375