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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study aims to evaluate the antibacterial profile of *Brysocarpus coccineus* and *Zanthoxylum piperitum* in Nigeria. The antibacterial efficacy of methanolic leaf extracts of *Brysocarpus 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 *Brysocarpus 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 6.25 mg/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 a significant difference in the activity of the extract, while

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that of *Z. piperitum* showed that F-cal is less than F-tab. meaning there is no significant difference in 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: Brysocarpus coccineus; Zanthoxylum piperitum; Minimum Inhibitory Concentration (MIC); antibacterial activity.

1. INTRODUCTION

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

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

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

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Samples

The plant material used for the various test in this work is *Zanthoxylum piperitum* and *Brysocarpus coccineus.* The leaves were obtained from different locations in Nsukka; Obukpa (6° 54' 0" North, 7° 24' 0" East), Ehandiagu-Eha- Alumona (6° 50' 0" North, 7° 27' 0" East) and Orba (Latitude: 6°51'14.76", Longitude: 7°27'45") during the dry season. *Brysocarpus coccineus* was got from an open farm-land in Ehandiagu-Eha- Alumona (6° 50' 0" North, 7° 27' 0" East), and *Zanthoxylum piperitum* was got from a thick forest in Orba area ((Latitude: 6°51'14.76", Longitude: 7°27'45"). The leaves were identified by Mr Ozioko from Bioresource Development and Conservation Programme, Nsukka. These plants were then air-dried in the lab at room temperature for about 5 days. The dried parts were subsequently reduced to a fine powder using an electric waring blender (model 51BL30).

2.2 Methanol Extraction of Anti-Microbial Substances from *Brysocarpus coccineus and Zanthoxylum piperitum* Leaves

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

accordingly and stored in the refrigerator while the research lasted.

2.3 Percentage Yield of Extract

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

Percentage yield (% yield) =

$$\frac{\text{mass of dried extract (g)}}{\text{Mass of pulverized plant material (g)}} \times 100 \quad \ldots. \quad (1)$$

2.4 Phytochemical Analysis

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

2.4.1 Test for alkaloids

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

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

2.4.2 Test for tannins

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

2.4.3 Test for flavonoids

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

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

2.4.4 Test for saponins

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

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

2.4.5 Test for glycosides

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

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

2.4.6 Test for steroids

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

2.4.7 Test for terpenoids

A quantity of 5 ml of methanol extract of plant sample was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added

to form a layer and observed for a reddish brown colouration at the interface.

2.4.8 Test for carbohydrates

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

2.4.9 Test for resins

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

2.5 Screening for Antimicrobial Activity

2.5.1 Test microorganisms

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

2.5.2 Preparation of 0.5 McFarland standards

Briefly, 1.175 g of Barium chloride crystal ($BaCl_2$) were weighed out and dissolved in 100 ml distilled water to give 1.175% barium chloride solution ($BaCl_2.2H_2O$). A 1 ml of concentrated tetraoxosulphate IV acid (H_2SO_4) was added to 99ml of distilled water to make 1% H_2SO_4 solution. Furthermore, 9.95 ml of 1% H_2SO_4 solution was mixed with 0.05 ml of 1.175% Barium chloride solution to make 0.5 McFarland's standard. This was used to standardize the test isolates.

2.5.3 Preliminary screening for the antibacterial activities of the extracts using the agar diffusion technique

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

2.5.4 Determination of the Minimum Inhibitory Concentration (MIC) using the broth micro dilution technique

Approximately 5 ml of Muller Hinton broth were pipetted into six tubes for each organism. A 0.5 ml of the diluted extract was added to the first tube and twofold serial dilution was done to get different concentration. A loopful of the test isolates were inoculated into the various tubes using a sterile loop-full picked from an 18hrs old broth culture of the organism, and incubated for 24 hrs at 37ºC. The least dilution of extract that showed no activity, when compared to the control, was taken as the minimum inhibitory concentration (MIC).

2.6 Statistical Analysis

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

3. RESULTS

3.1 Percentage Yield

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

Table 1. Percentage yields of extracts

Methanol extracts yields	%
B. coccineus	5.6
Z. piperitum	4.0

3.2 Phytochemical Analysis

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

3.3 Antimicrobial Activity of Extracts

3.3.1 The preliminary tests

The Methanol extract had a great spectrum of activity against the test organisms at different concentration. However, *Salmonella typhi* showed no activity in the two plants extracts respectively. Clinical isolate of *Bacillus subtilis*

Table 2. Phytochemical components of methanol extract of *Brysocarpus coccineus* and *Zanthoxylum piperitum*

	B. coccineus	*Z. piperitum*
Alkaloid	-	++
Saponins	+++	+
Tannins	-	++
Glycosides	++	++
Flavonoids	++	+
Carbohydrate	+	-
Steroid	++	+
Resin	++	++

Key: + Low concentration; ++ Medium concentration; +++ High concentration; - No activity

Table 3. Antibacterial activity of extracts of *Brysocarpus coccineus*

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

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
B. subtilis	11	9	9	8.5	8	7
S. aureus	10	10	9.5	9	0	0
E. coli	11	10	9.5	9.2	8.9	8.4
P. aeruginosa	11	10	9	0	0	0
K. pneumonia	12	11	10	9.2	8	7
S. typhi	0	0	0	0	0	0

Table 5. Antimicrobial activity of the extract on the type culture

Test organism	*B. coccineus*	*Z. piperitum*
S. aureus	15	13
E. coli	16	10
P. aeruginosa	-	-

Table 6. Comparison of antibacterial activity of the leaf extracts of *B. coccineus, Z. piperitum* and ciprofloxacin

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

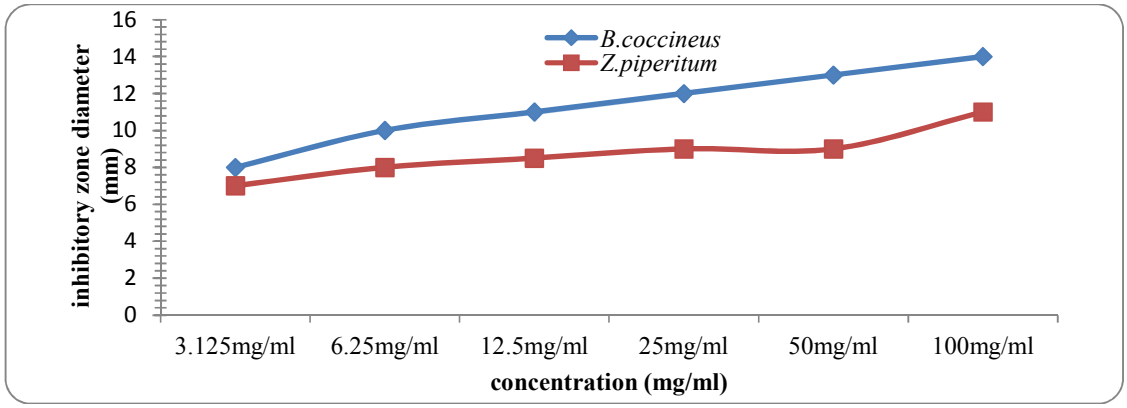

Fig. 1. Antibacterial activity of the two plants extract on *B. subtilis*

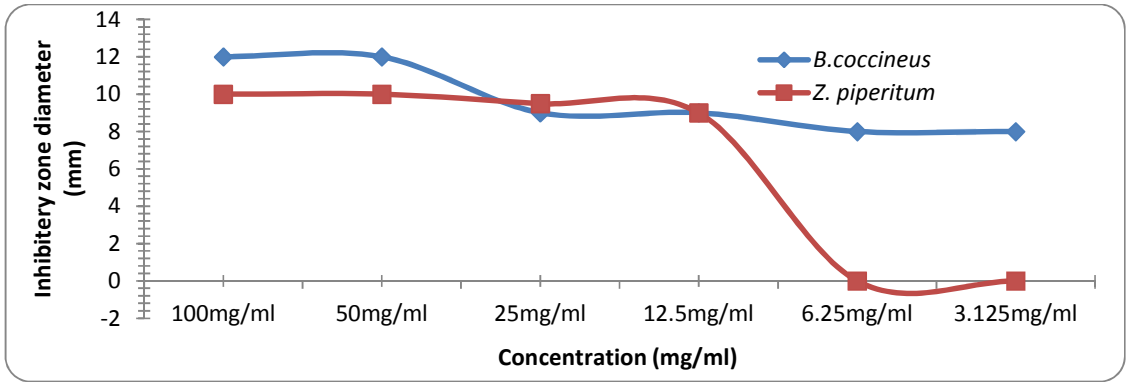

Fig. 2. Antibacterial activity of the two plants extract on *S. aureus*

and *Escherichia coli* were most susceptible as they displayed the widest zones of inhibition against the test organisms, using the two plant extracts respectively (Tables 3 and 4). *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed moderate zones of inhibition at different concentration in *Brysocarpus coccineus* when compared with *Zanthoxylum piperitum* extract, where *Staphylococcus aureus* showed zones of inhibition on the concentration of 100 mg/ml (10 mm), 50 mg/ml (10 mm) and 25 mg/ml (9 mm) respectively. *Pseudomonas aeruginosa* showed activities in higher concentrations except at 6.25 mg/ml and 3.125 mg/ml respectively. *Klebsiella pneumonia* showed no zones of inhibition with *Brysocarpus coccineus* extract, while *Zanthoxylum piperitum* extract inhibited it at different concentration.

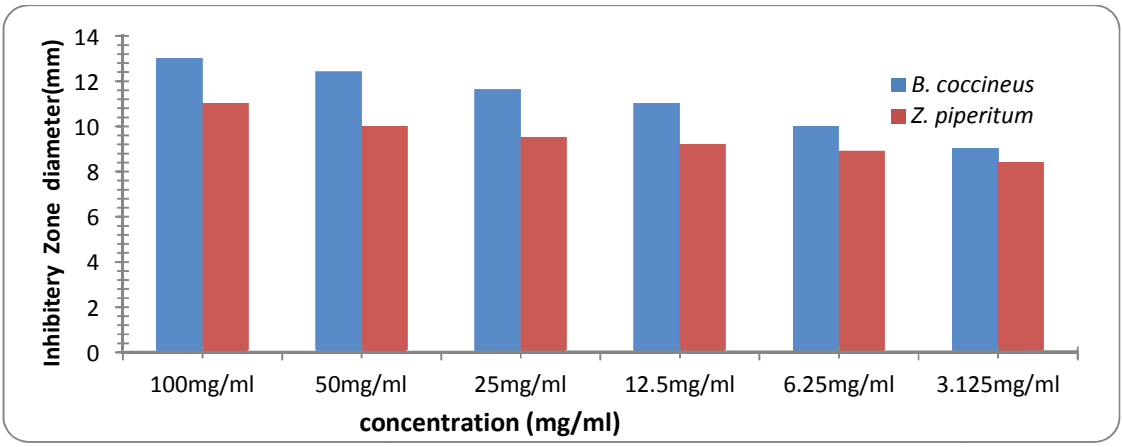

Fig. 3. Antibacterial activity of the two plants extract on *Escherichia coli*

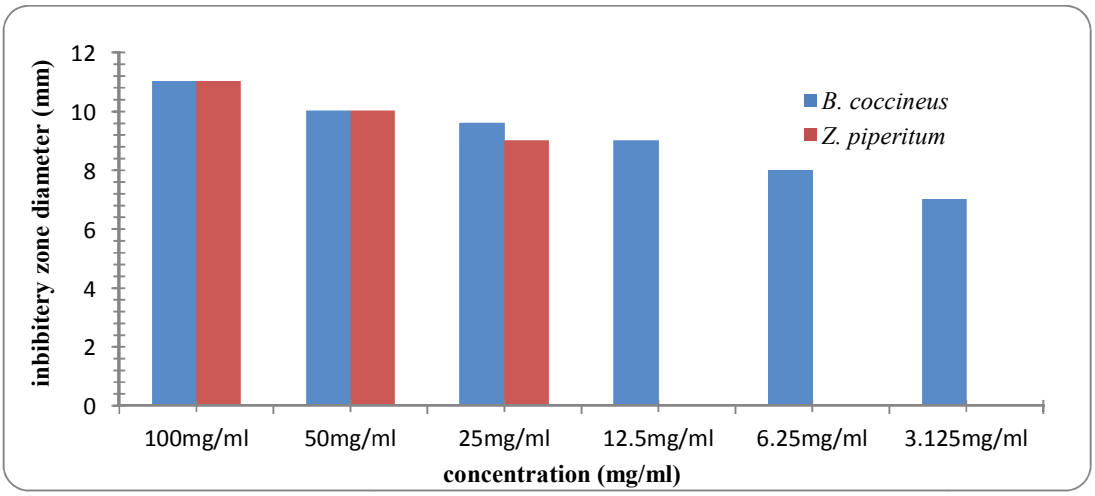

Fig. 4. Antibacterial activity of the two plants extracts on *Pseudomonas aeruginosa*

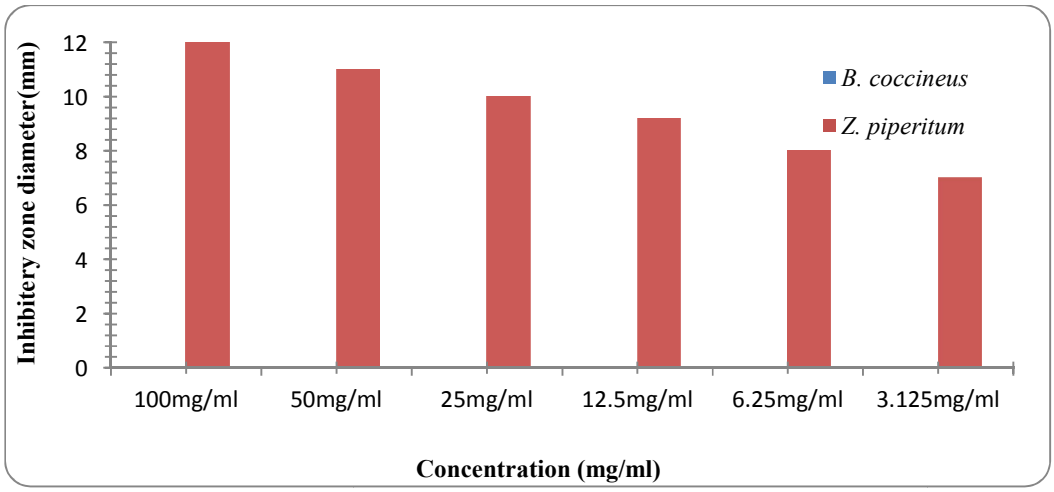

Fig. 5. Antibacterial activity of the two plants extract on *Klebsiella pneumonia*

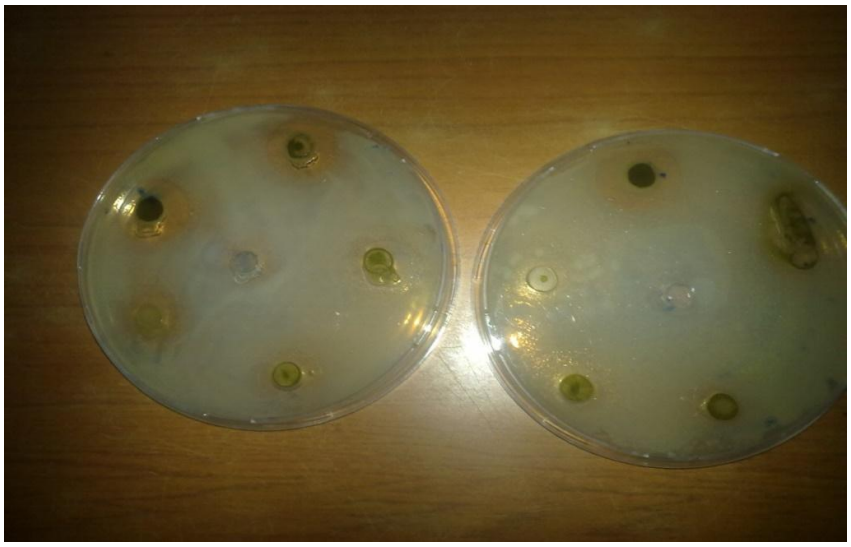

Plate 1. Inhibition zone diameter of *Brysocarpus coccineus* and *Zanthoxylum piperitum* methanol extract on microorganisms

Table 7. Results of MIC for methanolic extract of *Brysocarpus coccineus* leaves

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

Key: - = No growth; + = Growth

Table 8. Results of MIC for methanol extract of *Zanthoxylum piperitum* leaves

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

Key: - = No growth; + = Growth

Table 9. Interpretation the MIC results

Test organisms	**Minimum inhibitory diameter (mg/mL)**	
	B. coccineus	***Z. piperitum***
S .aureus	12.5	6.25
K. pneumonia	0	25
B. subtilis	6.25	6.25
S. typhi	0	0
P. aeruginosa	12.5	25
E. coli	6.25	25

Table 10. 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		

Table 11. 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		

3.3.2 Antimicrobial activity of the extracts on the type culture

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

3.3.3 Comparison of the two extract on test isolates

The two plants extract were checked for their activity on the test organisms. The extract with the highest activity on a particular plant extract was determined. In Figs.1, 2 and Fig. 3, it was found that *B. coccineus* had the highest activity IZD (14 mm, 12 mm and 13 mm). In Fig. 5, where they were tested against *K. Pneumonia, Z. piperitum* had the highest activity IZD (12 mm).

3.3.4 Comparison of the antibacterial activity of the leaf extracts of *B. coccineus*, *Z. piperitum* and ciprofloxacin

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

3.4 Results of the Statistical Analysis for *B. coccineus* Extract

At 95% Significant Level, F tabulated = 3.29,
F calculated =3.6482. Since Fcal> Ftab; this shows that the treatment means were not equal i.e. there was a significant difference between the treatment means. The least significant difference (LSD) value between the means is 3.0009. However, in the ranking of the means, the difference between the consecutive means is < LSD value. This shows that there was no significant difference between the means.

3.5 Results of the Statistical Analysis for *Z. piperitum* Extract

At 95% confidence interval, F tabulated was 2.25 and F calculated was 6024. Since Fcal < Ftab; this shows that the treatment means were not equal hence there was a significant difference between the treatment means. The least significant difference (LSD) value between the means equals 5.5287. However, in ranking of the means, the difference between the consecutive means was >LSD value. This means that there was a significant difference between the means.

4. DISCUSSION

The methanol extracts of *Brysocarpus coccineus* and *Zanthoxylum piperitum* leaves were evaluated for their antimicrobial activity against the following organisms clinical isolates of *E.coli, S. aureus, B. subtilis, P. aeruginosa, K. pneumonia, S. typhi* and typed strain of *S. aureus* (ATCC 2278), *E .coli* (ATCC 2127), *and P. aeruginosa* (*UCH* 2078) . The percentage yield of the methanolic extractions determined showed 5.6% for *Brysocarpus coccineus* and 4% for *Zanthoxylum piperitum*. This revealed that methanol extracts gave more yield of *Brysocarpus coccineus* than with *Zanthoxylum piperitum* leaves. Since the composition of methanol is the same, a plausible explanation for the greater extraction power of methanol in *B. coccineus* over *Z. piperitum* may be as a result of the composition of the plant material and as a result of methanol to extract both polar and non-polar compounds that constitute the plant's structure.

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

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

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

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

5. CONCLUSION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adebayo EA, Ishola OR, Majolagbe ON. Evaluation of the methanol extracts of *Ficus exasperata* stems, leaf and root for phytochemistry and antimicrobial activities. African Journal of Plant Science. 2009;3(12):283-287.
2. Wazis CH, Anuka JA, Timothy SY, Zezi AU, Mohammed GT, Hussaini IM. Acute toxicity and in-vivo effects of leaf extracts of *Brysocarpus coccineus* shum & thonn in pregnant rat uterus. Journal of Applied Pharmaceutical Science. 2012;2(12):130-136.
3. Gill LS. Ethnomedical uses of plants in Nigeria. Uniben Press, Benin City. 2000;15-65.
4. Ejeh SA, Onyeyili P, Abalaka SE. Anti-diarrhea activity of the aqueous root bark extract of *Byrsocarpus coccineus* on castor

oil-induced diarrhea in Wistar rats. Veterinary World. 2017;10(7):743-747.
5. Chorpaka P, Nuchnipa N, Hataichanoke N. Antifungal activity of the essential oil extracted from *Zanthoxylum piperitum* seeds against *Aspergillus flavus.* Chiang Mai J. Sci. 2017;44(2):584-594.
6. Walla FM, Bushra HS, Reem NI, Mohammed BH. Antibacterial activity of *Zingiber officinale* (Ginger) against clinical bacterial isolates. South Asian Journal of Research in Microbiology. 2019;3(2):1-7.
7. Essien AD, Essiet GA, Akuodor GC, Akpan JL, Chilaka KC, Bassey AL, Ezeokpo BC, Nwobodo NN. Pharmacological evaluation of the aqueous stem barks extract of *Bombax buonopozense* in the relief of pain and fever. African Journal of Pharmacy and Pharmacology. 2016;10(5):59-65.
8. Akindele AJ, Adeyemi OO. Anxiolytic and sedative effects of *Brysocarpus coccineus* Schum and Thonn. (Connaraceae) extract. International Journal of Applied Research in Natural Plant. 2007;3(1):28-36.
9. Oke JM, Hamburger MO. Screening of some Nigerian medicinal plant for antioxidant activity using 2-2-Diphenyl-picryl-hydrazyl radical. African Journal of Biomedical Research. 2002;5(1-2):77-79.
10. Akindele AJ, Adeyemi OO. Anxiolytic and sedative effects of *Brysocarpus coccineus* Schum and Thonn. (Connaraceae) extract. International Journal of Applied Research in Natural Plant. 2010;3(1):28-36.
11. Akindele AJ, Adeyemi OO. Analgesic activities of the aqueous leaf extract of *Brysocarpus coccineus*. Nigerian Journal of Health and Biomedical Science. 2006;5(1):43-46.
12. Ahamadu AA, Hassan HS, Abubakar MU. Flavonoids glucoside from *Brysocarpus coccineus* leaves Schum and Thonn Connaraceae). Africa Journal of Traditional, Complimentary and Alternative Medicines. 2007;4(3):257-260.
13. Yamazaki E, Inagaki M, Kurita O, Inoue T. Antioxidant activity of Japanese pepper (*Zanthoxylum piperitum* DC.) fruit. Food Chemistry. 2007;100:171–177.
14. Chorpaka P, Nuchinpa N, Hataichanoke N. Antifungal activities of the essential oil extracted from *Zanthoxylum piperitum* seeds against *Aspergillus flavus*. Chiang Mai Journal of Science. 2017;44(2):584-594.
15. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd Edition. Chapman and Hall, London. 1998;302. ISBN: 0-412-57270-2.
16. Gislene G, Locatelli J, Freitas P, Silva G. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian Journal of Microbiology. 2000;31(4):1517-1590.
17. Jain S, Sherma R. Antimicrobial activity of pyrrolizidine alkaloids from *Heliotropium ellipticum*. Journal of Chemistry and Pharmacology. 1986;35(8):3487-3489.
18. Ajali U. Chemistry of biocompounds. 1st Edition, RhyceKerex Publishers, Enugu. 2004;97-118.
19. Bobbarala V, Katikala P, Naidu K, Penumajji S. Antifungal activity of selected plant extracts against pathogenic fungi *Aspergillus niger* F2723. Indian Journal of Science and Technology. 2009;2(4):0974-6846.
20. Seo YY, Sang-Hyun L, Bui HT, Hae-Dong J, Young HK. Antioxidant and anti-osteoporosis activities of chemical constituents of the stems of *Zanthoxylum piperitum*. Molecules. 2018;23-457.

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