PHYTOCHEMICAL SCREENING AND BACTERIOCIDAL ACTIVITY OF ETHANO FLOWER EXTRACTS OF *BORRERIA VERTICILLATA* AND *VOCANGAAFRICANA*

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ABSTRACT

This study evaluated the bactericidal activities of the ethanolic crude extracts of the flowers of *Borreria verticillata* and *Vocanga africana*. Air dried and ground flowers were macerated in 70% ethanol separately and extracted using a separating funnel. The crude extracts obtained were subjected to antibacterial assays against *Staphylococcus aureus* and *Escherichia coli* using the Agar well diffusion sensitivity test. Ciprofloxacin (a broad spectrum antibiotic) was used as positive control in a concentration of 10mg/ml and the crude extracts were tested in the concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml. Results obtained showed no activity on *E. coli* by the two plant crude extracts while an MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of 6.25 mg/ml and 25 mg/ml respectively were obtained on *S. aureus* by *Vocanga Africana* in contrast to an MIC and MBC of 25 mg/ml and 25 mg/ml respectively on *S. aureus* by *Borreria verticillata*. This shows that the two plants flower extracts had no antibacterial activity on *E. coli* but had antibacterial activity on *S. aureus*. The phytochemical contents of the two flower extracts showed the same profile of carbohydrates, alkaloids, flavonoids, tannins, saponins, triterpenes, sterols and cardiac glycosides. The results indicate the possibility of sourcing new pharmacophores from the flowers of the plants against pathogenic bacteria.

Keywords: *Borreria verticillata, Vocanga africana, Escherichia coli, Staphylococcus aureus,* antibacterial.
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INTRODUCTION

Plants therapeutic essence is secondary metabolites, known as phytochemicals. These organic chemical substances are stored in matured cells of the various organs, such as roots, stems, leaves, flowers, fruits and seeds [1].

Borreria verticillata L belongs to the family Rubiaceae; it is a perennial weedy herb and a very common tropical plant used in traditional pharmacopeia to recover cutaneous infections [2, 3, 4]. The parts of the plant commonly used are the stem, roots and leaves. The crushed leaves of *B. verticillata* are applied in scarification in case of anaemia while the stem bark decoction is used orally against infectious diseases including sexually transmitted diseases [5, 6, 7]. Tea forms of the root decoction are used in the treatment of gonorrhoea and leucorrhea [8].

Vocanga africana Stapf is a mosophytic perennial shrub found in the tropical rain forest and guinea savanna woodland belt belonging to the family Apocynaceae [1]. The parts of the plant commonly used are the root, stem, leaves and fruits. The root and leaf decoctions of the plant have been implicated in folk medicine for the treatment of malaria, diarrhea, infant convulsion, insane persons and heart ache [1, 9, 10].

This study evaluated the phytochemical composition and the antibacterial activities of the ethanol flower extracts of the plants towards establishing their cidal value against pathogenic bacteria.

MATERIALS AND METHODS

Whole plant of *Borreria verticillata* was collected from the old Jos Road, Zaria while *Vocanga africana* was collected from the Department of Biological Sciences Botanical Garden, Ahmadu Bello University, Zaria. The plants were taken to the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where they were identified and assigned voucher numbers - 672 and 1467 respectively by Mr. Gallah Umar.

Extraction of plant materials

The flowers of *Borreria verticillata* and *Vocanga africana* were separately washed, dried under shade for one week and then ground to powder form using mortar and pestle. 118g of *B. verticillata* and 104g of *V. africana* were soaked separately in 1 litre of 70% ethanol for 7 days after which the extracted materials were filtered and concentrated on a water bath at 60°C [11].

Phytochemical screening

The extracts were screened for the presence of bioactive agents according to the methods described by Sumathi [11] and Trease and Evans [12].

Collection of test organisms

Pure isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. The bacteria were sub-cultured and maintained on Nutrient agar slant at 4^{o}C in a refrigerator.

Susceptibility test

The agar well diffusion technique was used as described by Prescott [13]. Four (4) wells of 8mm diameter each were made on inoculated nutrient agar using a sterile cork borer. The wells were filled with different concentrations of the extract; 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml

allowed to diffuse for about 2 hours. Discs of 10mg of ciprofloxacin (a broad spectrum antibiotic) were used as positive control. The test plates were incubated at 37^{o}C for 24 hr. and the observed zones of inhibition measured with a transparent metre rule.

Minimum inhibitory concentration

Concentrations of 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.5625 mg/ml were tested for each extract. The lowest concentration of each extract showing no visible growth after 24 hours was recorded as the minimum inhibitory concentration (MIC).

Minimum bactericidal concentration

The inoculums from the pure culture tubes containing different concentrations of the extracts showing no visible growth of the organisms from the MIC test were sub-cultured on sterile Nutrient Agar plates and incubated at 37^{o}C for 24 hr. The lowest concentration of each extract without growth was noted as the minimum bactericidal concentration (MBC).

RESULTS AND DISCUSSION

The phytochemical profile of the two test extracts, showed the presence of the same bioactive agents namely saponins, tannins, alkaloids, flavonoids, triterpenes, sterols, carbohydrates and cardiac glycosides while free and combined anthraquinones and steroids were absent in the two extracts (Table 1). This similarity in phytochemical profile could be due to the fact that both plant materials used were flowers. Duru and Onnyedineke [1] reported similar findings of the phytochemicals in the ethanolic extracts of dried seeds of *Vocanga africana* which included phenols, phlobatanins, anthranoids and starch. Also Ushiel and Adamu [4] reported the same phytochemicals in the leaf extract of *Borreria verticillata* in which hexane, ethyl acetate, acetone, chloroform and methanol were used in the extraction. This indicates that both the flowers and leaves have similarity in their phytochemical content. This similarity is not surprising given that flowers are indeed modified leaves.

The zones of inhibition against *E. coli* and *S. aureus* are shown in Table 2. No inhibition on the growth of *E. coli* by both extracts was observed. However, *S. aureus* was susceptible to both extracts with a dose dependent inhibition ranging from 12.5 mm- 15 mm for *B. vericillata* and 11 mm- 17 mm for *V. africana*. This is in line with the findings of Ushiel and Adamu [4] who reported that ethyl acetate, hexane, acetone, chloroform and methanol leaf extract of *Borreria verticillata* have inhibitory effect on *S. aureus* and Duru and Onyedineke [1] who reported that hot and cold absolute ethanol extracts of the seeds of *Vocanga africana* had antimicrobial activity against *S. aureus.*

The MIC of the extracts against *S. aureus* was 6.25mg/ml for *V. africana* and 25mg/ml for *B. verticillata*, as shown in Table 3. The MBC of the extracts against *S. aureus* were 25mg/ml for both *V. africana* and *B. verticillata*.

Both extracts exhibited antibacterial activity against *S. aureus*. This may be due to the presence of some and the same phytochemicals and also the fact that *S. aureus* is a Gram positive bacterium in contrast to *E. coli* which is a Gram negative bacterium. The contrast may also be attributed to the possibility of low concentration of the extracts tested against *E. coli* and also the absence of some phytochemicals such as free and combined anthraquinone and sterols. The sensitivity test shows that the extracts were less potent than the drug of choice ciprofloxacin which was used as the positive control in the study, having a zone of inhibition of 45mm and 51mm against *E. coli* and *S. aureus* respectively. The reduced efficacy of the extracts relative to the antibiotic may be due to the fact that the extracts are in the crude form and require further purification in order to isolate the active ingredient(s) contained in them.

CONCLUSION

The results of this study showed that the ethanol extracts of the flowers of *Borreria verticillata* and *Vocanga africana* possess antibacterial agents against *Staphylococcus aureus* but none against *Escherichia coli.*

ACKNOWLEDGEMENTS

Our appreciation to Mallam Kabir of the Department of Pharmacognosy and Drug Development, Faculty of Pharmacy and Mallam Adamu Shittu of the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Samaru, Zaria for technical support on laboratory analysis. Our unending gratitude goes to Mr. and Mrs. C. M. Anele for financial support.

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Table 1: Phytochemical screening of the ethanol extracts of the flowers of *Borreria verticillata* and *Vocanga africana.*

Phytochemical Test	***Borreria verticillata***	***Vocanga Africana***
Carbohydrate	+	+
Free anthraquinone	-	-
Combined anthraquinone	-	-
Alkaloids	+	+
Flavonoids	+	+
Tanins	+	+
Saponins	+	+
Triterpenes	+	+
Steroids	-	-
Sterols	+	+
Cardiac glycosides	+	+

Table 2: Antibacterial activity of the ethanol extracts of the flowers of *B. verticillata* and *V. africana* on *E. coli* and *S. aureus*.

Extract	**Test organism**	**Average zones of inhibition[mm]**			
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
B. verticillata	*S. aureus*	15	10.5	12.5	0
V. africana	*S. aureus*	17	15	11	0
B. verticillata	*E. coli*	0	0	0	0
V. africana	*E. coli*	0	0	0	0

Table 3: MIC of the ethanol extracts of the flowers of *B. verticillata* and *V. africana* on *S. aureus.*

Test organism	**Extract**	**Concentration[mg/ml]**				
		25	12.5	6.25	3.125	1.5625
	B. verticillata	-	+	+	+	+
S. aureus	*V. Africana*	-	-	-	+	+