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Functional ingredients and antimicrobial activity of *Borrerria verticilata* root extract

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Abstract

Background and objective: Medicinal plants have been used in traditional medicine to treat several diseases and infections. This study aimed to identify the active ingredients in *Borrerria verticilata* root extract for the synthesis of new antimicrobial drugs.

Materials and methods: Roots of *Borerria verticillata* were collected from Ucha village, Makurdi local government of Benue State, Nigeria. For extraction, hexane, chloroform, ethyl acetate, methanol, and water were used as solvent. The roots were air-dried and powdered before extraction. The extracts were qualitatively tested for alkaloids, flavonoids, tannins, saponins, glycosides, cardiac glycosides, terpenes, steroids, and phenol. Antimicrobial potency of the extracts against some Gram-positive and Gram-negative organisms, and some fungi implicated in dermatophytic infections were studied. Agar well diffusion and broth dilution methods were used to determine the minimum inhibitory concentration and minimum bactericidal/fungicidal concentration.

Results and conclusion: The yield of the extracts ranged from 1.5% for water to 3.5% for hexane as solvent. The phytochemicals in the root extracts were included to flavonoids, terpenes, saponins, and glycosides. The microbial inhibition zone, minimum inhibitory concentration, and minimum bactericidal/fungicidal concentration ranged from 0 to 21.67 mm, 8 to 512 mg/ml, and 256 to 512 mg/ml, respectively. This study revealed that the root extract of *Borerria verticillata* plant has an antimicrobial effect against multidrug-resistant bacteria and also antifungal potential. Therefore, it can be used in food and cosmetics industries.

Keywords: Antimicrobial potency, *Borrerria verticilata*, Phytochemical ingredients, Root extract

1. Introduction

Microbial resistance has dramatically increased in the past three decades due to the introduction of antimicrobial agents that have created an immense chemical problem in treating infectious diseases. Other than ineffectiveness of the antibiotics against multi-drug resistant bacteria, the declining budget for the treatment of infectious diseases is of concern [1]. Due to the emergence of drug resistance in human pathogens, new prototypes of antimicrobial agents are needed to address this situation. It has led to the evaluation of plants as a source of potential chemo-therapeutic agents with antimicrobial activity. Medicinal plants contain some ingredients which can be used for therapeutic purposes or as precursor for synthesizing useful drugs [2]. They are rich in wide

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variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones [2-4], which have been used in traditional medicine to treat several diseases [5-8].

Many studies have shown that the medicinal plants and their extracts have multi-antimicrobial properties [9-12]. Currently, 25-50% of the pharmaceuticals are derived from plants, none of them is used for antimicrobial purposes [2]. The biological effects of these plants on prokaryotic and eukaryotic organisms have been highlighted in a few studies [13-14]. Plants have an almost infinite ability to synthesize the compounds with diverse bioactive properties that we cannot synthesize [15]. Many plant species have not been studied or described for potential medicinal value. Before the advent of orthodox medicine, most societies depended on traditional medicines for their healthcare needs. The traditional medicine men, in turn, relied on plants that had therapeutic values [16-17]. Introduction of novel drug compounds as plant-derived medicines has greatly contributed to human health [18]. The primary benefit of such medicines in healing is that they are relatively safer than synthetic alternatives within specific doses. Today, phytochemists and pharmaceutical companies depend on the medicinal plants to produce the medicines [19-20]. Most people in rural areas use herbs to treat several ailments because they have low cost and are available [21-24]. This study aimed to screen antimicrobial activity of the root extracts of *Borreria verticillata (BV)* against some dermatophytes and pathogenic bacteria that are multi-drug resistant. In addition, we identified the phytochemical constituents of BVR root by using different solvents.

2. Materials and methods

2.1. Sample collection and preparation

The BV plant readily available in rainy season was uprooted from the soil. They were collected from Ucha village, adjacent to the Federal University of Agriculture, Makurdi, Local Government of Benue State, Nigeria. They were transferred to the Department of Biological Sciences for identification and confirmation. Their identity was confirmed in the Botany unit (Nasarawa State University, Keffi). The roots were washed separately with running tap water to remove dirt before the drying process and were cut into small pieces of about 1-2 cm, followed by air-drying for 21 days and grounding into powder with a pistol and mortar.

2.2. Extraction of phytochemicals

Maceration was used for the extraction of BV roots according to Abubakar and Haque's method [25]. The macerated BV roots were air-dried for two weeks and milled into fine powder using a Thomas-Willey milling machine. Then, 100 g of powder was mixed with 250 ml of hexane solvent for four days. The mixture was subjected to filtration by Whatman filter paper number 1. The filtrates were dried in a water bath and weighed further with a *digital scale.* The procedure was repeated by other solvents including ethyl acetate, water, chloroform, and methanol. The extracts were stored in a desiccator at room temperature [26].

2.3. Phytochemical assay

2.3.1. Alkaloids

0.5 g of BV root extract was mixed with 5 ml HCl 2 M in a steam bath and filtered further. One ml of the filtrate was separately treated with a few drops of Mayer's reagent, Drangendoffs' reagent, and Wagner's reagent. The final solution was monitored for color change [27-30].

2.3.2. Tannins

0.5 g of BV root extract was boiled in a water bath for 5 min and left to cool down to room temperature. Then, a few drops of ferric chloride were added to 2 ml of the cooled solution (filtrate). The final solution was monitored for color change [27-30].

2.3.3. Glycosides

A small portion of each plant extract was placed in two separate test tubes. One of them was mixed with 0.1 M H_2SO_4, and another was mixed with 5 ml distilled water. The test tubes were heated for 45 min in a water

bath and left to cool down further. Then, 5 ml NaOH (2 M) was added to the cooled solutions [27-30]. Fehling solutions A (5 ml) and B (ratio1:1) were added to the test tubes and were left for 3 min. The extract in distilled water served as a control. The changes in reaction were observed and recorded.

2.3.4. Saponins

The froth and emulsion tests described by Harborne [31] were used to determine the presence of saponins. At first, 2 ml of each plant extract was mixed with 20 ml distilled water in a 100 ml beaker, followed by boiling and filtering with Whatman paper no. 1. For the froth test, 5 ml of the filtrate was diluted with 20 ml distilled water and shaken vigorously. Then, it was left for 30 min, and the result was recorded. For the emulsion test, two drops of olive oil were added to the frothing solution and shaken vigorously. The result was recorded. In order to remove the 'false positive" result, the blood hemolysis test was performed on the extract that frothed water.

2.3.5. Anthraquinones

0.5 g of BV root extract was shaken with 2 ml benzene and filtered with a filter paper. Then, 4 ml ammonia solution (10%) was added to the filtrate. The mixture was shaken, and the result was recorded [27-30].

2.3.6. Flavonoids

A lead acetate test was done by dissolving 0.5 g of the extract in 5 ml of distilled water. Then, 1 ml of lead acetate solution (10%) was added to the mixture, and the color formed was recorded. For the iron (III) chloride test, two drops of iron (III) chloride were added to 0.5 g of aqueous extract. The color change was recorded [27-30].

2.3.7. Terpenoids (Salkowski test)

At first, 0.5 g of each extract was dissolved in 2 ml chloroform and 1 ml concentrated H_2SO_4. The presence of terpenes in the sample was confirmed by the color change from red to yellow glow [28].

2.4. Microorganisms

Multi-drug resistant Gram-positive (*Staphylococcus aureus, Bacillus subtilis)* and Gram-negative (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi)* organisms were obtained from the Medical Microbiology and Parasitology unit of the Clinical Laboratory department of the Federal Medical Centre, Keffi. The fungi implicated in dermatophytic infections (*Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis, Epidermophyton floccosum*) were obtained from the Medical Microbiology and Parasitology department of the Ahmadu Bello University Teaching Hospital. The bacteria and fungi were maintained on nutrient agar and Sabouraud dextrose agar, respectively (Oxoid, UK). Twenty-four hours old pure cultures were prepared for use each time.

2.5. Antimicrobial assay

The procedure of Perez et al. [32] was used with a slight modification. The method involved the preparation of the culture medium and inoculation. An aseptic technique was used to avoid contamination [30,33]. The agar plates were inoculated by L-shaped glass by spreading a small volume (0.05-0.1 ml) of the liquid inoculum (sub-cultured nutrient broth). One microorganism was inoculated to one plate (ten plates for ten microbes). Five wells for hexane, chloroform, ethyl acetate, acetone, and methanol extracts and two for control were formed via a 4-cm diameter sterile cork borer. The plant extracts were diluted using dilution method, and added to the wells separately. Tetracycline and terbinafine were used as control, and added to the wells designed for control. The inoculated plates were left on the bench for about one hour to allow the extracts diffuse into the agar. The plates were aerobically incubated at 37 ºC for 24 h for the bacteria, and 72 h at 25 ºC for the fungi [33]. The diameter of the inhibition zones was measured by a vernier caliper and recorded in millimeters.

2.6. Minimum inhibitory concentration (MIC)

Suspension of microorganisms was made in sterile normal saline and adjusted to 0.5 MacFarland standard

(10^8 CFU/ml) [34]. Serial dilutions of the extracts were made from the stock solution at concentrations of 512, 256, 128, 64, 32, 16, 8, and 4 mg/ml [35]. Solutions of the extracts at different concentrations were assayed against the bacteria. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth [34].

2.7. Minimum bactericidal/fungicidal concentration (MBC/MFC)

MBC/MFC is the lowest MIC that shows no growth on Mueller Hinton Agar plate. Equal volumes of various concentrations of each extract and nutrient agar and Sabouraud dextrose agar separately were mixed in microtubes to make a 0.5 ml solution. Then, 0.5 McFarland standard suspension of each organism was added to the tube [35]. The tubes were incubated aerobically at 37 ºC for 24 h for bacteria, and at 25 ºC for 72 h for dermatophytes. The negative and positive controls containing the extract without inoculum and the inoculum without extract were prepared. MBC was determined by subculturing the test dilution on Mueller Hinton Agar and further incubation for 24 h. The highest dilution that yielded no single bacterial /fungal colony was taken as MBC/MFC [36,37].

2.8. Statistical analysis

The experiment was arranged in a randomized complete block design with three replications. Analysis was done using SPSS software version 25.0. The data was subjected to analysis of variance, and the means were compared by Duncan's test. Differences were significant at $P \leq 0.05$.

3. Results and discussion

Table 1 shows the nature and yields of different extracts of BV roots. The results indicated that hexane with brownish hard solid structure gave the highest yield of 3.5%, followed by chloroform with 2.2%, dark brown sticky solid texture. The aqueous solution resulted in light brown powder and the lowest yield of 1.5% (Table 1).

Table 1- Nature and yields of different solvents for extraction of *Borreria verticillate* roots

Solvent	Color of extract	Texture of extract	Yield (%)
Hexane	Brownish	Hard solid	3.50
Ethyl acetate	Dark brown	Sticky solid	2.20
Acetone	Light brown	Powder	2.05
Methanol	Light brown	Powder	1.64
Water	Light brown	Powder	1.50

Crude extracts of BV root contained glycosides, alkaloids, tannins, and anthraquinone. Flavonoids were present in methanolic and aqueous extracts. Terpenes were present in the extracts prepared by hexane, chloroform, and methanol. Saponins were not found in chloroform extract (Table 2).

Table 2- Phytochemical composition of *Borreria verticillata* roots extracts

		Extracts				
Phytochemicals	Reagents	HE	AE	CE	EAE	ME
Alkaloids	a) Wagners	-	-	-	-	-
	b) Mayer	-	-	-	-	-
	c) Drangedroff	-	-	-	-	-
Tannins	Solutions of extracts plus ammonia solution	-	-	-	-	-
Flavonoids	a) Lead acetate	-	+	-	-	+
	b) Ferric chloride	+	-	-	+	+

Anthraquinone	Extract in benzene plus ammonia solution	-	-	-	-	-
Terpenes	Extracts plus chloroform plus H_2SO_4	+	-	+	-	+
Saponins	a) Frothy test	+	+	-	+	+
	b) Emulsion test	+	+	-	+	+
Glycosides	Extracts plus dilute H_2SO_4 plus NaOH plus Fehiling solution	+	+	+	+	+

* - = absent; + = present

**HE = hexane extract; AE = aqueous extract, CE = chloroform extracts, EAE = ethyl acetate extracts, ME = methanol extracts.

Table 3 shows the diameter of inhibition zones of the microorganisms. Accordingly, *S. aureus*, *E. coli*, *S. typhi*, and *P. aeruginosa* were the most susceptible organisms with inhibition zone of 12.33 mm, 16 mm, 19 mm, and 21.67 mm, respectively, in the presence of ethyl acetate extract. Tetracycline, which served as control, had the highest impact on all the bacteria strains and a low impact on the dermatophyte of *E. Floccosum* with no impact on *M. canis, T. rubrum*, and *T. mentagrophytes.* Moreover, terbinafine had the highest impact on the dermatophytes and some bacteria except for *S, aureus, B. subtilis, S. typhi*, and *K. pneumoniae*.

Table 3- Inhibition zones (mm) of selected multiple drug-resistant bacteria and dermatophytes in the presence of *Borreria verticllata* roots extracts

Organism	HE	CE	EAE	AE	ME	TCN	TBN
Drug-resistant bacteria							
S. aureus	12.00^{b}	11.33^{b}	12.33^{b}	0.00^{c}	0.00^{c}	26.00^{a}	0.00^{c}
E. coli	$8.67^{c,d}$	10.67^{c}	16.00^{b}	15.67^{b}	10.00^{c}	25.33^{a}	6.67^{d}
B. subtilis	4.67^{d}	7.00^{c}	0.00^{e}	9.00^{b}	13.00^{a}	12.33^{a}	0.00^{e}
S. typhi	0.00^{c}	0.00^{c}	19.00^{a}	13.00^{b}	8.33^{b}	19.33^{a}	0.00^{e}
P. aeroginosa	8.00^{d}	14.00^{b}	21.67^{a}	$12.33^{b,c}$	$11.67^{b,c}$	19.33^{a}	5.33^{d}
K. pneumoniae	7.00^{d}	$8.00^{c,d}$	0.00^{e}	$11.00^{a,b}$	$10.00^{b,c}$	13.33^{a}	0.00^{e}
Dermatophytes							
M. canis	2.00^{a}	3.00^{a}	3.67^{a}	0.00^{a}	0.00^{a}	0.00^{a}	7.33^{a}
T. rubrum	1.67^{a}	$4.00^{b,c}$	$2.00^{b,c}$	6.33^{b}	6.00^{b}	0.00^{c}	22.00^{a}
E. floccosum	$1.67^{c,d}$	$2.33^{c,d}$	$6.00^{b,c}$	0.00^{d}	8.67^{b}	$3.33^{c,d}$	22.67^{a}
T. mentagrophytes	2.33^{b}	6.33^{b}	5.00^{b}	0.00^{b}	2.00^{b}	0.00^{b}	16.00^{a}

*Data are means of three replicates. The similar letters in each column show no significant difference ($p \leq 0.05$).

**HE= hexane extract; AE=aqueous extract, CE=chloroform extracts, EAE= ethyl acetate extracts, ME= methanol extracts, TCN= tetracycline, TBN= terbinafine

Investigation of MIC results revealed that the extracts prepared by hexane and chloroform showed the highest inhibition against *P. aeruginosa* and *T. mentagrophytes*, respectively (Table 4). Furthermore, hexane extract inhibited *S. aureus, E. coli*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa, T. mentagrophytes,* and *T. rubrum*. Chloroform extract showed no inhibition against *E. floccosum,* and *M. canis*. All of the dermatophytes except for *M. canis* were inhibited by ethyl acetate extract. In addition, *E. coli* and *S. typhi* were not inhibited by ethyl acetate extract. Similar results in bacterial inhibition were observed for aqueous extract. Except for *M. canis,* all of the microorganisms were inhibited by methanol extract.

MBC/MFC of the extracts ranged from 256 to 512 mg/ml. According to Table 5, no growth was observed for the microorganisms in the presence of hexane except for *S. typhi* and *E. floccosum.* Chloroform extract successfully surpassed the viability of microorganisms. However, *E. coli*, *S. typhi*, *T. mentagrophytes,* and *E. floccosum* were inhibited at

concentrations of 256, 512, 256, and 512 mg/ml, respectively. Ethyl acetate extract showed the highest MBC/MFC so that it inhibited the viability of all microorganisms (no colony was observed on the plate). Aqueous extract surpassed the viability of *B. subtilis*, *T. rubrum*, and *M. canis*. MBC of 512, 256, and 512 mg/ml was achieved for *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*, respectively, by methanolic extract.

Medicinal plants contain active compounds with biological activity; some of them are responsible for the odor, pungencies, and color of the plants, while others make the plant appropriate for culinary, medicinal, or poisonous virtues [27,30, 38-39]. Screening of phytochemicals in BV roots showed the presence of some secondary metabolites with medicinal activity as well as exhibiting physiological activity. These secondary metabolites are responsible for the plant's anti-allergic, anti-inflammatory, and antimicrobial properties [40]. They are also antioxidants and free radical scavengers that prevent cell damage, have strong anticancer activity, and protect the cell against carcinogenesis [41]. Therefore, they are a candidate for use in the medical, pharmaceutical, and cosmetic industries [42]. For instance, saponins as a secondary metabolite are used in traditional medicine preparations [43]. Extraction of secondary metabolites from BV roots yielded various phytochemicals. However, the highest amounts were obtained by using hexane. Tannins, flavonoids, saponins, terpenes, and glycosides were present in some of the extracts, while tannins, anthraquinones, and alkaloids were not found in all the extracts. The absence of tannins might be due to the plant's genetics. In comparison, the phytochemicals mentioned in this study were reported by Muhammad et al. in BV roots [38]. It has been reported that the phytochemicals could have overt larvicidal efficacies against *Culex quinquefasciatus* and some bionematicides [44]. It agrees with the finding of Ushie et al. in evaluating BV stem bark [30]. In comparison, flavonoids, saponins, and tannins were present in ethyl acetate and methanolic extract of the BV plant, and anthraquinones and steroids were not found in a study of Kontagora et al. [45]. However, Karou et al. stated that alkaloids are the main components of the plants belonging to the Rubiaceae family, including BV [46]. Flavonoids, alkaloids, tannins, and saponins are responsible for the insecticidal effects of the plants and their toxicity to other animals [47-48]. BV roots showed antimicrobial activity at particular concentrations. It is in agreement with the findings of Olateju and Samuel. [43], in which BV roots showed high antimicrobial activity. Furthermore, Abdullahi-Gero et al. [49] pointed out that BV plants possess antimicrobial action at different concentrations depending on the bacteria species. Neto et al. [50] showed that methanolic extract of BV roots has high activity against six different strains of *P. aeruginosa*, even against the strains identified as resistant to a broad spectrum of antibiotics, including the last generation of quinolones and cefalosporins. Jeff-Agboola and Onifade reported the inhibitory action of BV root against dermatophytes. In their study, different root extracts had antifungal effects on *Aspergillus flavus*, *A. parasiticus*, and *A. ochraceus*, which are responsible for the deterioration of stored seeds and grains [51]. Cheesbrough [52] pointed out that the active antimicrobial compounds diffuse from the disc into the medium, and susceptible organisms are inhibited at a distance from the disc by the antimicrobial agents (BV root extracts in our study). This statement is comparable to the known potent antiobiotics including tetracycline and fulcin in suppression of both Gram-positive and Gram-negative bacteria, and fungi [30].

The genus of Borreria has demonstrated antimicrobial activity against multi-drug resistant bacteria and even fungi [53]. It is also worth noting that MIC and MBC/MFC depended on the extraction method. It suggests that these plant extracts inhibit bacterial growth without necessarily killing the bacteria. Most traditional preparations do not have specific concentrations. Therefore, a large quantity of extracts may be used in traditional medicine to treat patients [54].

Table 4- Minimum inhibitory concentration (mg/ml) of *Borreria verticillata* root extracts against selected multi-drug resistant bacteria and dermatophytes

Extract	*S. aureus*	*E. coli*	*B. subtilis*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*	*T. mentagrophytes*	*T. Rubrum*	*E. floccosum*	*M. canis*
HE	512[a]	512[a]	256[b]	-	128[b]	32[b]	128[a]	512[a]	-	-
CE	128[b]	128[b]	256[b]	512[a]	128[b]	32[b]	8[b]	256[b]	-	-
EAE	256[b]	-	512[a]	-	512[a]	256[a]	64[a,b]	256[b]	256[b]	-
AE	256[b]	-	512[a]	-	512[a]	256[a]	64[a,b]	256[b]	256[b]	512[a]
ME	256[b]	512[a]	512[a]	256[b]	128[b]	64[a,b]	8[b]	512[a]	512[a]	-

*Data are means of three replicates. The similar letters in each column show no significant difference ($p \leq 0.05$).
**HE= hexane extract; AE=aqueous extract, CE=chloroform extracts, EAE= ethyl acetate extracts, ME= methanol extracts, TCN= tetracycline, TBN= terbinafine

Table 5- Minimum bactericidal/fungicidal concentration (mg/ml) of *Borreria verticillata* root extract against some selected multi-drug resistant bacteria and dermatophytes

Extract	*S. aureus*	*E. coli*	*B. subtilis*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*	*T. mentagrophytes*	*T. rubrum*	*E. floccosum*	*M. canis*
HE	-	-	-	256[b]	-	-	-	-	512[a]	-
CE	-	256[a,b]	-	512[a]	-	-	256[a,b]	-	512[a]	-
EAE	-	-	-	-	-	-	-	-	-	-
AE	256[a,b]	512[a]	-	512[a]	256[a]	256[a,b]	512 [a]	-	512[a]	-
ME	512[a]	-	-	-	256[a]	512[a]	-	-	-	-

*Data are means of three replicates. The similar letters in each column show no significant difference ($p \leq 0.05$).
**HE= hexane extract; AE=aqueous extract, CE=chloroform extracts, EAE= ethyl acetate extracts, ME= methanol extracts, TCN= tetracycline, TBN= terbinafine

Therefore, the practitioners can be sure of their therapeutic effect at high doses.

4.Conclusion

The present investigation confirms the folkloric use of BV roots as indigenous medicine for treating some bacterial- and fungal-associated diseases. The plant contains important bioactive substances (phytochemicals) including flavonoids, terpenes, saponins, and glycosides. Cosmetics and pharmaceutical industries could exploit these chemicals. Our results could be served as platform for isolation and elucidation of these bioactive compounds.

5. Conflict of interest

The authors declare that they have no conflict of interest.

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