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Full Length Research Article

PHYTOCHEMICAL ASSAY AND ANTIBACTERIAL APPRAISAL OF METHANOLIC LEAF EXTRACTS OF *BORRERIA VERTICILLATA* AND *AGERATUM CONYZOIDES* AGAINST MDR NOSOCOMIAL PATHOGENS

*Otu J.U.[1], Bassey E.E.[1], Usang I.I.[2] and Obiaje J.U.[1]

[1]Department of Microbiology, University of Cross River State, P.M. B. 1123, Calabar, Nigeria
[2]Department of Public Health, Ahmad Bello University, Zaria, Kaduna State, Nigeria

*Corresponding Author Email Address: josephotu@unicross.edu.ng or joeotu14@gmail.com

ABSTRACT
In a bid to find solution to the global menace of antibiotic resistance, the current work attempts to explore the phytochemical composition and validate the antibacterial potential of methanolic leaf extracts of *B. verticillata* and *A. conyzoides* against nosocomial pathogens. The test organisms namely *Klebsiella pneumoniae, Acinetobacter baumannii* and *Enterococcus faecalis* were collected from Microbiology laboratory, Nigerian Navy Reference Hospital, Calabar, Nigeria. The bacteria were authenticated by standard microbiological methods. Multi-drug resistance attribute of the organisms was determined by Kirby Bauer disc diffusion technique. Methanol was used as solvent for plants' extraction by the aid of Soxhlet apparatus. Phytochemical evaluation was carried out using standard protocol and subjected to GC-MS analysis. The *in vitro* antibacterial potentials of the plants' extracts were investigated against the bacterial isolates using agar well diffusion technique. Results revealed the presence of saponins, alkaloids, cardiac glycosides, flavonoids, steroids, tannins, phenols and volatile oils in the methanolic leaf extract of *B. verticillata*. Also, extract of *A. conyzoides* indicated the presence of alkaloids, cardiac glycosides, saponins, flavonoids, phenols, volatile oils and tannins. GC-MS investigation showed the detection of hexadecenoic acid, methyl ester, 11-0ctadecanoic acid, methyl stearate, 9-octadecanamide, 1,2-benzenedicarboxylic acid and butyl-2-ethylhexyl ester in *A. conyzoides*. The major components detected in *B. verticillata* leaf extract were 1.2-15,16-dieoxyhexadecane, 1,2-benzenedicarboxylic acid, butyl 2-ethyhexyl ester, hexadecenoic acid, 2,3-bis (trimethyl islyl) oxyl propyl ester, 9-octadecenamide, 2-ethylbutyric acid and eicosyl ester, 3-tetradecanol. It was observed that *K. pneumoniae* was resistant to amoxicillin, ofloxacin and clindamycin with a percentage drug resistance rate of 3(21.43%). *A. baumannii* was resistant to eight (8) antibiotics including vancomycin, amoxicillin+clavulanic acid and had percentage drug resistance rate of 8(53.43%). *E. faecalis* was resistant to five (5) antibiotics including vancomycin, amoxillicin and ciprofloxacin while the percentage drug resistance rate was 5(35.71%). The results established the fact that the bacterial isolates exhibited multidrug-resistance. *B. verticillata* leaf extract inhibited bacterial growth producing zone sizes ranging from 7.7 to 17.4mm. Antibacterial activity of *A. conyzoides* against the organisms revealed lesser zone sizes (9.6 to 14.1mm) with *A. baumannii* having the least zone size (12.6mm) at 100% concentration. Therefore, it can be concluded that *B. verticillata* and *A. conyzoides* present remarkable potential of producing significant plant-derived pharmaceuticals against multidrug-resistant nosocomial pathogens.

Keywords: *Multidrug-resistant (MDR), GC-MS, nosocomial, phytochemical, antibacterial*.

1.0 INTRODUCTION

Borreria verticillata is a clambering and climbing, perennial shrubby, false button weed belonging to the family Rubiaceace, which has immense potential as a medicinal herb. (Ushie *et al*., 2013). It is widely distributed in tropical areas in Africa, Asia, and South America. The plant has many other designations by which it is referred to globally (Campos *et al*., 2014). This plant species is popularly called whitehead broom, southern larra flower and shrubby false buttonwood in English language. In West Africa countries, different tribes and ethnic groups have their unique names for this weed (Abdullahi-Gero *et al*., 2014; Andrioli *et al*., 2014). In Nigeria, it is known as *karya garma* in Hausa, *wantiyo kporou* in Tiv, *irawa-ile* in Yoruba and *abia-ikana* in Ibibio (Ushie and Adamu, 2010; Aremu *et al*., 2019).

B. verticillata is a common weed in West Africa countries though it is reputed for its use in traditional medicine in Asia, Africa, Latin America, and West Indies (Balde *et al*., 2015). In West Africa region, its decoction (extraction from the upper part) is applied topically on the skin for management of skin related ailments such as *Tinea versicolor* (eczema), *Tinea capitis* (ring worm), *Pityriasis versicolor*, skin itches, psoriasis, scabies, and various infectious dermatitis (Balde *et al*., 2015). Some authors report that the tea from the roots of *B. verticillata* is employed in the management of leucorreas and blenorreas (Peixoto-Neto *et al*., 2018). In Brazil, its leaves and flowers infusion are used as analgesic and antipyretic (Kontagora *et al*., 2017). The blend from the roots is utilized as emetic and its broad leaves are employed as antidiarrheal, against haemorrhoids and erysipelas.

In West Indies, decoction from *B. verticillata,* which is usually called Alpha Marrow or Wild Margaret, is employed to manage high blood pressure and as an abortifacient (Balde *et al*., 2015). Also, it is prepared with *Cuscuta* and *Zebrina Schnizlein* employed in the treatment of amenorrhea and against diabetes and dysmenorrhea (Aremu *et al*, 2019). In the Northern Senegal, it is used against leprosy and skin related diseases. It is used as purgative against paralysis, gonorrhoeal sores, leprosy, bilharzia, furuncles, infantile hyperpnexia and ulcers (Ushie *et al*., 2013; Bello *et al*., 2017, 2019). An infusion of the leaves is also used in treating various skin diseases in Nigeria and some parts of eastern Africa (Sofowora, 1993). Essential oil extracted from the leaves and other part of plants has been shown to inhibit the growth of *Culex quinquefasciatus, Staphylococcus aureus, Escherichia coli* and

Candida albicans (Kontagora, 2017).

Ageratum conyzoides is a tropical plant that is very common in West Africa, Australia, some parts of Asia and South America (Iwuagwu *et al.,* 2019). It is an erect, annual, branched, slender, hairy, and aromatic herb, which grows to approximately 1 m in height. The stems and leaves are covered with fine white hairs, the leaves are stalked, ovate, 4-10 cm long and 1-5 cm wide, with tip and base somewhat pointed and with round-toothed margins long (Kamboj and Saluja, 2018). The flowers are purple to white, less than 6 mm across and arranged in close terminal inflorescences. The fruit is black and are easily dispersed while the seeds are photoblastic and often lost within 12 months (Couett *et al*., 2016). *A. conyzoides* has been used in various parts of Africa, Asia, and South America for curing various diseases. Githen, in his review (Githens, 1948), listed the uses of the plant as purgative, febrifuge, for ophthalmia, colic, treatment of ulcers, and wound dressing. The antineuralgic and the antipyretic properties of the plant were also indicated in a review on ëMedicinal plants from Senegalí (Kerharo and Adam, 1974). In some African countries, the plant has been popularly used for the treatment of skin diseases, wound healing, mental and infectious diseases, headaches, and dyspnea (Sarfo-Antwi *et al*., 2018). It is also used in traditional medicine for its anti-asthmatic, antimicrobial, antispasmodic and haemostatic effects, uterine troubles, pneumonia by rubbing them on the chest of the patient (Sharma and Sharma, 2015). In Cameroon, it is a local remedy for *craw-craw* (Kumar and Jain, 2018).

Microorganisms including many types of pathogenic bacterial species cause infectious diseases. Scientific studies have implicated bacterial pathogens (e.g., *Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli,* etc.) as etiologic agents of most hospital-acquired (nosocomial) infections (Iroha *et al.,* 2012). These infections have been reported to account for approximately 66.67 % of all deaths in tropical countries (Adejobi *et al.,* 2021). The use of antibiotics has been the treatment method for these infections. However, bacterial strains that are resistant to many used drugs belonging to different classes of antibiotics (multidrug resistance) have emerged leading to increase in treatment failures (Ibanga *et al*., 2020). Currently, antibiotic resistance has become a global concern, hence, the shift back to the use of traditional medicine (Farhan *et al*., 2019). This new drive has birthed methodical and empirical screening of many plants for their medicinal properties and antimicrobial effect in an attempt to provide more effective and efficient treatment alternatives (Akanbi *et al*., 2018).

The medicinal value of some plants lies in some chemical substances (phytochemicals) they contain, which are secondary metabolites that produce definite physiological actions in the body. These important bioactive constituents include but not limited to alkaloids, tannins, flavonoids, glycosides, terpenes, and steroids, anthraquinones, phenolic compounds (Ahmed *et al*., 2020). Many phytochemicals belonging to several chemical classes have been shown to demonstrate inhibitory effect on many types of microorganisms *in vitro* and *in vivo* (Aiyegoro *et al*., 2019). Recently, secondary metabolites previously with unknown pharmacological activities have been examined as sources of medicinal and therapeutic agents (Almagboul *et al*., 2018).
In developing countries such as Nigeria, there has been a gradual revival of interest in the use of medicinal plants due to their availability, accessibility, cheap, safe, and less adverse effect when compared to synthetic drugs (Bello *et al*., 2019). It has also been reported that the use of medicinal plants in the treatment of ailments has been on the increase in Nigeria as many orthodox drugs are adulterated and therefore ineffective in the treatment of diseases (Aremu *et al*., 2019). Therefore, the present study appraises the phytochemical composition and antibacterial effect of methanolic leaf extracts of *B. verticillata* and *A. conyzoides* against multidrug-resistant (MDR) nosocomial pathogens isolated in Nigeria Navy Reference Hospital, Calabar, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Plant collection and Identification of Plant Material

Fresh, healthy, and young leaves of *B. verticillata* and *A. conyzoides* were collected from Cross River National Park and washed with distilled water. The leaves were jointly identified by the Departments of Botany, University of Calabar and Plant Science and Biotechnology, University of Cross River State, Nigeria

Borreria verticillata L.

Ageratum conyzoides L.

2.2 Preparation of Plant Material

The leaves were cleaned and dried under room temperature for 7 days and then ground well to a fine powder. About 500 g of dry powder was extracted with methanol (80%) at 70°C by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 hrs., and the methanolic extract was then filtered and kept in a hot air oven at 40°C for 24 hrs. to evaporate the methanol from it. A dark brown residue was obtained. The residue was kept separately in airtight containers and stored in a deep freezer.

2.3 Preliminary Phytochemical Screening of the Extracts

Preliminary phytochemical screening was carried out on methanolic leaf extracts of *B. verticillata* and *A. conyzoides* for secondary metabolites such as saponins, alkaloids, carbohydrates, flavonoids, phenols, proteins, terpenoids, tannins, phlobatannins, volatile oils and anthraquinones as described by Sofowora (1993) and Evans (2009).

2.4 Gas Chromatography-Mass spectrometry (GC-MS)

Gas chromatography mass spectroscopy (GC-MS), a hyphenated system which is a very compatible technique and the most used technique for identification and quantification purposes was employed. The unknown phytochemical compounds in the complex organic mixture were determined by interpretation and by matching the spectra with reference spectra (Gavamukulya *et al*., 2015).

2.4.1 GC-MS Analysis

GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Schimadzu GCMS-QP2010, employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) as carrier gas at a constant flow of 1ml/ minute and a sample injection volume of 1 µl which was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9-minute isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total run time was 30 min. The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectra of the components were compared with the database of spectrum of known components stored in the GC-MS library using National Industrial Securit Program (NISP) Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. The retention time, which is the time elapsed between injection and elution was also used in differentiating compounds. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.

2.5 Source of test organisms

Three (3) stock clinical isolates of *Klebsiella pneumoniae, Acinetobacter baumannii* and *Enterococcus faecalis* were collected from the Microbiology Laboratory, Nigerian Navy Reference Hospital, Calabar, Cross River State, Nigeria. The organisms were authenticated by colonial characteristics, microscopy and biochemical reactions as described by Koneman *et al*. (2005) and Cheesbrough (2006). The organisms were preserved at 2 - 8°C until required. Purity of the organisms was checked at regular intervals.

2.6 Evaluation of multidrug resistance of test organisms

Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method (Liofilchem, Teramo, Italy) on Mueller–Hinton agar (MHA) plates. During testing, the susceptibilities of the clinical bacterial isolates to amoxicillin (AMO; 10 µg), amikacin (AMI; 30 µg), ofloxacin (OFL; 20 µg), ceftazidime (CEF; 30 µg), amoxicillin+clavulanic acid (AMO+CLA; 20 µg), erythromycin (ERY; 15 µg), clindamycin (CLI; 2 µg), ciprofloxacin (CIP; 5 µg), gentamicin (GEN; 10 µg), sulfamethoxazole/trimethoprim (SXT; 23.75/1.25 µg), vancomycin (VAN; 10 µg), rifampicin (RIF; 5 µg), imipenem (INI: 10 µg) and nalidixic acid (NAA: 30 µg) were determined. The discs were aseptically mounted on the emulsified and diffused isolates in the Petri dishes, labelled and inverted at 37°C for 24 h, for zones of inhibitions reports after 24 h using Vernier Caliper in accordance with Clinical Laboratory Standard Institute Chesbrough, (2010) and CLSI (2020). Multidrug resistance is taken as resistant to three (3) or more groups of antibiotics.

2.7 Antibacterial susceptibility assay using methanolic leaf extracts

The standardization of culture was carried out as described by Bakar and Thomsberg (1983) and Clinical and Laboratory Standards Institute (CLSI, 2020). Briefly, an 18 h culture of the test organism was suspended in a sterile universal bottle containing nutrient broth. Normal saline was added gradually to it to compare the turbidity to that of 0.5 McFarland standard corresponding to approximately 10^8 cells/ml. This was then diluted to produce10^6 cells/ml that was used in the experiments. To carry out the antibacterial susceptibility test, the method described by Emeruwa (1982) was used. One milliliter (1ml) of test organism (10^6 cells/ml) was inoculated into Petri dishes (90 mm diameter), then 19 ml molten Mueller Hinton agar (MHA) at 45°C added, and the plates shaken gently for even mixing of the contents. The agar was allowed to solidify on a flat bench. Wells (6 mm diameter and 4 mm deep) were punched in the agar with the aid of a sterile cork borer. The plant extract was reconstituted by dissolving 200 mg of each extract in 1 ml distilled water and then pipetted (0.5 ml) into holes bored from the agar. 0.5 ml of the pure solvent (methanol) was used as negative control, and 0.5 ml of 50 mg/ml solution of Tigecycline antibiotic was used as positive control. The plates were left on a flat bench for 1 h to dry, before incubating at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of zones of growth inhibition in triplicates and the mean of three results taken for both the test organisms and controls.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Phytochemical Constituents of Plant Extracts

Table 1 shows the phytochemical composition of *A. conyzoides* and *B. verticillata* methanolic leaf extracts. It is depicted that saponins, alkaloids, cardiac glycosides, flavonoids, steroids, tannins, phenols and volatile oils are present in the methanolic leaf

extract of *B. verticillata* while anthraquinones is not detected. In the methanolic extract of *A. conyzoides,* alkaloids, cardiac glycosides, saponins, flavonoids, phenols, volatile oils and tannins were present, and anthraquinone and steroids were absent.

3.1.2 GC-MS analysis of methanolic leaf extracts of *B. verticillate* and *A. conyzoides*

The total ion chromatogram (TIC) of the methanolic extract of *A. conyzoides,* showing GC-MS profile of the compounds identified is presented in Figure 1. The peaks in the chromatogram were integrated and compared with the database of spectrum of known components stored in the GC-MS NISP library. Phytochemical assay by GC-MS analysis revealed the presence of different esters, fatty acids, heterocyclic compounds among others. Seventeen (17) peaks were generated. The detailed tabulations of GC-MS evaluation of the methanolic extract are given in Table 2. From the analysis, 17 phytochemical compounds were marched and identified. The major constituents were at peak 1 (peak area 55.82%) hexadecenoic acid and methyl ester, peak 4 (peak area 18.24%) 11-0ctadecanoic acid and methyl ester, peak 6 (peak area 9.74%) methyl stearate, peak 10 (peak area 2.47%) 9-octadecanamide and peak 2 (peak area 2.04%) 1,2-benzenedicarboxylic acid and butyl-2-ethylhexyl ester. Other phytochemical compounds had less than 2% composition by peak area.

Figure 2 shows the total ion chromatogram (TIC) of methanolic leaf extract of *B. verticillata* revealing GC-MS credentials of the identified phytochemical substances. Results indicate that a total of 23 phytochemical compounds were marched and identified. The major components were at peak 22 (peak area 23.87%) 1.2-15,16-dieoxyhexadecane, peak 9 (1,2-benzenedicarboxylic acid and butyl 2-ethyhexyl ester, peak 19 (peak area 11.74%) hexadecenoic acid, 2,3-bis (trimethyl islyl) oxyl propyl ester, peak 15 (peak area 9.01%) 9-octadecenamide, peak 23 (peak area 3.02%) 2-ethylbutyric acid and eicosyl ester, peak 12 (peak area 2.63%) 3-tetradecanol, peak 18 (peak area 2.45%) hexadecenoic acid, 2,3-bis [(trimethyl silyl) oxy] peopyl ester and peak 21 (peak area of 2.01%) Z,Z,Z-8,9-epoxyeicosa 5,11,14-trienoic acid (table 3).

3.2 Authentication of test organisms

Table 4 represents the morphological and biochemical characteristics of clinical bacterial isolates. According to the result, *K. pnuemoniae* was Gram negative rod with capsule, nonmotile, catalase positive, oxidase negative, doesn't hydrolysis esculin and no nitrate reduction. *A. baumannii* was coccobacilli (in pairs), Gram negative with capsule, hydrolysis esculin, nonmotile, doesn't reduce nitrate, catalase positive and oxidase negative. *E. faecalis* was observed as cocci cells, Gram positive, no capsule, nonmotile, hydrolysis esculin, reduces nitrate, catalase negative and oxidase negative.

Table 1: Preliminary phytochemical compounds of methanolic leaf extracts of *B. verticillata* and *A. conyzoides*

S/N	Metabolite	Methanolic Extracts	
		B. verticillata	*A. conyzoides*
1.	Saponins	+	++
2.	Alkaloids	+	++
3.	Cardiac Glycosides	+++	++
4.	Flavonoids	+	+
5.	Anthraquinones	-	-
6.	Steroids	+	-
7.	Tannins	+++	++
8.	Phenols	++	+
9.	Volatile oils	++	+

- = Not present; + = low presence; ++ = moderately present; +++ = high concentration of metabolite

3.3 Antibiotics susceptibility profile of clinical bacterial isolates

Table 5 represents the antibiotic susceptibility pattern of the test organisms. According to the result, *K. pneumoniae* was resistant to amoxicillin, ofloxacin and clindamycin with mean zones of inhibition 12.4mm, 10.1mm and 0mm, respectively. *K. pneumoniae* was susceptible to the other antibiotics (vancomycin – 22.2mm, imipenem – 28.2mm, ceftazidime – 24.2mm, amoxicillin+clavulanic acid – 20.3mm, rifampicin – 21.3mm, erythromycin – 20.2mm, nalidixic acid – 19.6mm, ciprofloxacin – 20.5mm, amikacin-19.8mm) with varying mean zones of inhibition and intermediate to gentamycin (14.8mm) and sulfamethoxazole/trimethoprim (septrin) (15.5mm).

A. baumannii was resistant to eight (8) antibiotics namely vancomycin (no zone of inhibition), amoxicillin+clavulanic acid (11.2mm), amoxicillin (no zone of clearance), ofloxacin (12.5mm), ciprofloxacin (9.3mm), erythromycin (10.3mm), rifampicin (9.4mm) and clindamycin (no zone of inhibition) but sensitive to imipenem (25.5mm), ceftazidime (24.4mm), gentamycin (23.9mm), amikacin (24.6mm) and nalidixic acid (23.4mm); it was intermediate to septrin (14.2mm).

E. faecalis was resistant to five (5) antibiotics including vancomycin – 12.1mm, amoxicillin – 10.3mm, ciprofloxacin – 11.4mm, erythromycin – 12.5 and clindamycin – 9.4mm. The test organism was sensitive to imipenem (19.6mm), amoxicillin+cluvalanic acid (24.5mm), ceftazidime (26.2mm), gentamycin (27.1mm), amikacin (26.3mm), ofloxacin (22.5mm), nalidixic acid (25.2mm), rifampicin (19.5mm) and septrin (21.4mm). The results established the fact that the bacterial isolates are multidrug-resistant (resistance to three or more classes of antibiotics).

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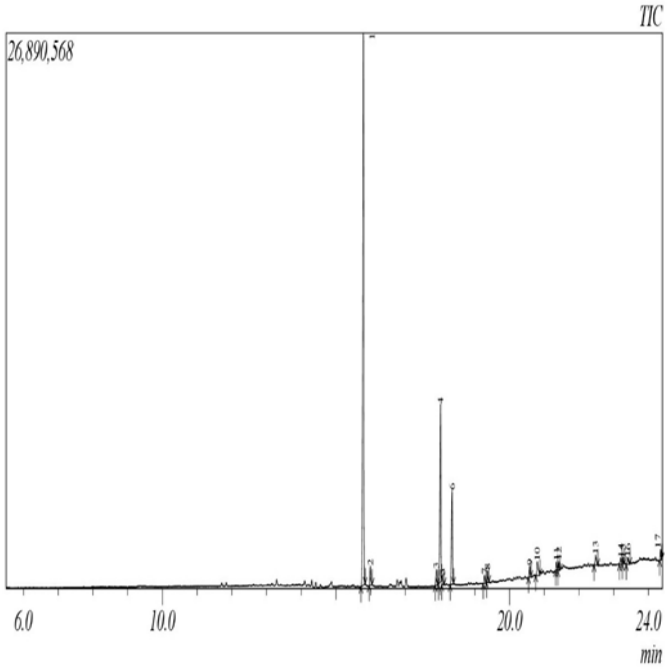

Figure 1: Total Ion Chromatogram (TIC) of methanolic leaf extract of A. conyzoides

Table 2: Phyto-components generated in the methanolic leaf extract of *A. conyzoides* by GC-MS peak report TIC

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	15.792	55.82	C17H34O2	270	94	Hexadecenoic acid, methyl ester
2	15.992	2.04	C20H30O4	334	92	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester
3	17.891	1.69	C17H30O2	266	91	7,10-Hexadecadienoic acid, methyl ester
4	18.009	18.24	C19H36O2	296	93	11-Octadecenoic acid, methyl ester
5	18.065	0.89	C19H36O2	296	83	11-Octadecenoic acid, methyl ester
6	18.342	9.74	C19H38O2	298	91	Methyl stearate
7	19.281	0.86	C24H50O	354	91	n-Tetracosanol-1
8	19.367	1.18	C22H45Cl3Si	442	87	Silane, trichlorodocosyl-
9	20.578	1.08	C21H42O2	326	89	Eicosanoid acid, methyl ester
10	20.800	2.47	C18H35NO	281	88	9-Octadecenamide, (Z)-
11	21.353	0.71	C21H44O	312	88	1-Heneicosanol
12	21.424	0.84	C26H54	366	89	Eicosane, 7-hexyl-
13	22.477	1.34	C23H46O2	354	87	Decanoic acid, methyl ester
14	23.205	0.75	C16H32	224	82	Cetene
15	23.267	0.43	C19H40	268	79	Heptadecane, 2,3-dimethyl-
16	23.408	0.81	C28H56O2	424	81	Heptacosanoic acid, methyl ester
17	24.354	1.10	C18H36O2	284	84	Hexadecanoic acid, 14-methyl-, methyl ester

SI = March factor based on library

3.4 Antibacterial activity of methanolic leaf extracts of *B. verticillata* and *A. conyzoides*

Table 6 shows the antibacterial activity of extract of *B. verticillata* against the clinical bacterial isolates. Result reveals that the mean zones of inhibition ranged from 17.4mm to 7.7mm. Individual sensitivity to plant extract indicates that *K. pneumoniae* had a mean zone of inhibition 16.1mm at 100mg/ml while *A. baumannii* obtained a mean zone size of 15.4mm at 100mg/ml concentration. Also, *E. faecalis* had 17.3mm as mean zone of clearance at 100mg/ml. It was observed that the mean zone size increased with increase in concentration of the extract. The extract demonstrated antibacterial activity (11.8mm) against *E. faecalis* at the lowest concentration (12.5mg/ml). The extract had no activity against *K. pneumoniae* and *A. baumanni* at the lowest concentration.

The antibacterial effect of methanolic leaf extract of *A. conyzoides* against the organisms is shown in Table 7. The mean zones of inhibition ranged from 15.2mm to 7.1mm at various concentrations. The highest zone size (15.2mm) was recorded against *E. faecalis* at 100mg/ml. The extract was least active against *A. baumannii* with 12.6mm as the highest activity against the organism. Again, *E, faecalis* was more susceptible to the extract than *K. pneumoniae* and *A. baumannii*. In comparison, *B. verticillata* demonstrated more antibacterial activity than *A. conyzoides*.

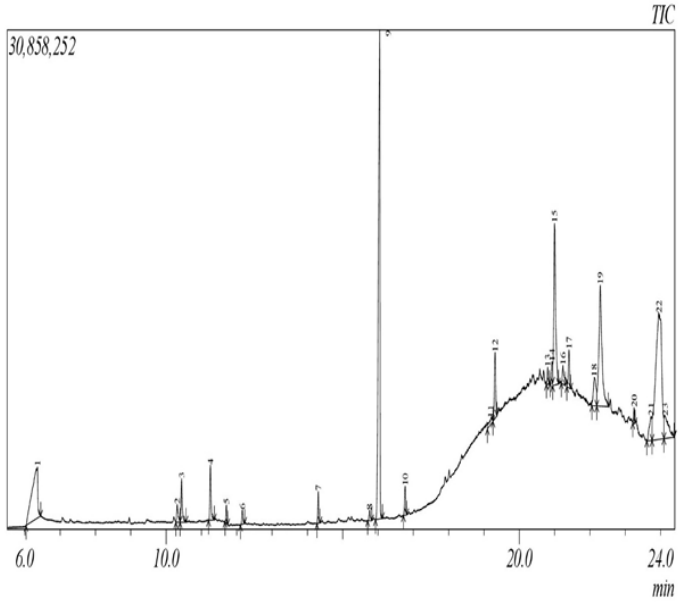

Figure 2: Total Ion Chromatogram (TIC) of methanolic leaf extract of B. verticillata.

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Table 3: Phyto-components generated in the methanolic leaf extract of *B. verticillata* by GC-MS peak report TIC

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	6.356	9.49	C7H6O2	122	95	Benzoic acid
2	10.316	0.85	C9H14O7	234	89	Citric acid, trimethyl ester
3	10.440	1.69	C14H22O	206	95	Phenol, 2,4-bis(1,1-dimethylethyl)-
4	11.255	1.96	C12H20O7	276	81	Triethyl citrate
5	11.707	0.50	C13H26	182	93	3-Tridecene, (Z)-
6	12.160	0.46	C12H20O7	276	94	Triethyl citrate
7	14.309	0.85	C12H24	168	92	1-Dodecene
8	15.763	0.43	C16H32O2	256	88	Tetradecanoic acid, 12-methyl-, methyl ester
9	16.044	23.04	C20H30O4	334	87	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester
10	16.767	0.92	C15H32O2	244	92	1,3-Propanediol, 2-dodecyl
11	19.190	0.72	C6H6N4O3S	214	46	5-Nitrothiophene-2-carboxaldehyde semicarbazone
12	19.313	2.63	C14H30O	214	87	3-Tetradecanol
13	20.805	0.52	C18H34O2	282	80	Oleic Acid
14	20.929	0.87	C10H16O3	184	64	1-Oxaspiro [2.5] octane-2-carboxylic acid, ethyl ester
15	20.999	9.01	C18H35NO	281	92	9-Octadecenamide, (Z)-
16	21.237	0.95	C18H35NO	281	83	9-Octadecenamide, (Z)-
17	21.405	1.56	C19H38	266	91	1-Nonadecene
18	22.122	2.45	C25H54O4Si2	474	67	Hexadecenoic acid, 2,3-bis[(trimethylsilyl) oxy] propyl ester
19	22.295	11.74	C19H40O2Si	328	72	Hexadecenoic acid, trimethylsilyl ester
20	23.248	0.45	C22H44	308	89	1-Docosene
21	23.735	2.01	C21H34O3	334	56	Z,Z,Z-8,9-Epoxyeicosa-5,11,14-trienoic acid, methyl ester
22	23.951	23.87	C16H30O2	254	70	1,2-15,16-Diepoxyhexadecane
23	24.125	3.02	C26H52O2	396	64	2-Ethylbutyric acid, eicosyl ester

SI = March factor based on library

Table 4: Morphological and biochemical characterization of clinical bacterial isolates

Morphological/ Biochemical parameters	*A. baumannii*	*K. pneumoniae*	*E. faecalis*
Shape	Coccobacilli (pairs)	Rod	Cocci (monococci)
Gram stain	-	-	+
Motility	Non-motile	Non-motile	Non-motile
Catalase	+	+	-
Oxidase	-	-	-
Indole	-	-	-
Simmons citrate	+	+	-
Urea, Christensen	-	+	-
Nitrate reduction	-	-	+
Methyl red	-	-	-
Voges Proskauer	-	-	+
Esculin hydrolysis	-	+	+
TSI acid:			
Slant	Red	Yellow	Yellow
Gas	-	+	+
Butt	Red	Red	Red
H_2S	-	-	-
Haemolysis	-	-	-
Presence of capsule	+	+	-

- = Negative, + = Positive, H_2S = Hydrogen sulphide, += Present, - = Absent

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Table 5: Antibiotics susceptibility profile of clinical bacterial isolates

	Zones of inhibitions (mm)/test organisms		
Class/Antibiotics	***K. pneumoniae***	***A. baumannii***	***E. faecalis***
Beta-lactams			
Vancomycin	22.2±0.4 (S)	0±0.0 (R)	12.1±0.5 (R)
Amoxicillin	12.4±0.6 (R)	0±0.0 (R)	10.3±0.3 (R)
Imipenem	28.2±0.3 (I)	25.5±0.4 (S)	27.6±0.6 (S)
Amoxicillin+clavulanic acid	20.3±0.5 (S)	11.2±0.4 (R)	24.5±0.3 (S)
Ceftazidime	24.2±0.3 (S)	24.4±0.6 (S)	26.2±0.5 (S)
Aminoglycosides			
Gentamycin	14.8±0.4 (I)	23.9±0.5 (S)	25.1±0.4 (S)
Amikacin	27.8±0.6 (S)	24.6±0.4 (S)	26.3±0.3 (S)
Quinolones			
Ciprofloxacin	20.5±0.5 (S)	9.3±0.4 (R)	11.4±0.5 (R)
Ofloxacin	10.1±0.2 (R)	12.5±0.4 (R)	22.5±0.5 (S)
Nalidixic acid	19.6±0.3 (S)	23.4±0.6 (S)	25.2±0.3 (S)
Macrolides			
Erythromycin	20.2±0.4 (S)	10.3±0.6 (R)	12.5±0.5 (R)
Rifampicin	21.3±0.6 (S)	9.4±0.4 (R)	19.5±0.5 (S)
Sulphonamide			
Septrin	15.5±0.5 (I)	14.2±0.4 (I)	21.4±0.6 (S)
Lincosamide			
Clindamycin	0±0.0 (R)	0± 0.0(R)	9.4±0.6 (R)
% drug resistance rate	**3(21.43)**	**8(53.33)**	**5(35.71)**

% = Percentage, R = Resistant, S = Sensitive, I = Intermediate

E. faecalis antibiotic susceptibility

A. baumannii antibiotic susceptibility

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Table 6: Antibacterial effect of methanolic leaf extract of *B. verticillata*

	Concentrations (mg/ml) and zones of inhibitions (mm)					
						Controls
Isolate	100	50	25	12.5	M	TGC
K. pneumoniae	16.1±0.6	13.2±0.4	7.7±0.3	0±0.0	0	42
A. baumannii	15.4±0.5	13.4±0.2	10.2±0.0	0±0.0	0	39
E. faecalis	17.3±0.4	15.4±0.4	13.1±0.5	11.8±0.6	0	45

M = Methanol, TGC = Tigecycline, mg/ml = milligram per mil, mm = millimetre

Table 7: Antibacterial effect of methanolic leaf extract of *A. conyzoides*

	Concentrations (mg/ml) and zones of inhibitions (mm)					
						Controls
Isolate	100	50	25	12.5	M	TGC
K. pneumoniae	14.1±0.2	12.2±0.5	0±0.0	0±0.0	0	42
A. baumannii	12.6±0.3	7.1±0.6	0±0.0	0±0.0	0	39
E. faecalis	15.2±0.5	12.7±0.5	9.6±0.4	0±0.0	0	45

M = Methanol, TGC = Tigecycline, mg/ml = milligram per mil, mm = millimetre

DISCUSSION

The deployment of medicinal plants in the treatment of different diseases, including hospital-acquired bacterial infections, has received promising and increasing attention in recent years, especially in developing countries (Aremu *et al*., 2019). This is due to the fact that plants produce natural products that have been known to be effective against pathogens, with few side effects compared to commercial antibiotics (Ohemu and Fajoyomi, 2022). The use of plants as treatment alternative has become even compelling in the face of antimicrobial resistance and multidrug resistance development by bacterial pathogens (Mutalib *et al*., 2015). Many empirical investigations have demonstrated that phytoconstituents may meaningfully add to the formulation of new and potent medications that can modify bacterial resistance as an alternate and complementary method of addressing microbial resistance (Dzotam and Kuete, 2017; Wintola *et al*., 2021; Fagbemi *et al*., 2022). This study evaluated the phytochemical composition and antibacterial effect of methanolic leaf extracts of *B. verticillata* and *A. conyzoides* against multidrug-resistant (MDR) nosocomial pathogens.

Methanol was selected as extraction solvent, because it is one of the best solvents used for the extraction of antimicrobial substances (Odeleye *et al*., 2014; Waheed *et al*., 2019). Moreover, methanol polarity ensured the extraction of polar and moderately polar active compounds from plants against microorganisms like terpenoids, tannins, flavones, and polyphenols, flavones and terpenoids (Shivam *et al*., 2022).

Results of preliminary phytochemical screening of methanolic leaf extracts of *B. verticillata* and *A. conyzoides* indicated the presence of saponins, alkaloids, cardiac glycosides, flavonoids, steroids, tannins, phenols and volatile oils in the extract of *B. verticillata* (table 1). Ushie *et al*. (2013) and Rufa'i *et al*. (2020) reported the presence of these phytochemicals in *B. verticillata*. These secondary metabolites have been reported to have considerable antibacterial activities (Balde *et al* 2015; Waheed *et al*., 2019). However, the relative composition of phytochemicals obtained in this study diverges from phytochemicals in *B. verticillata* leaf extract as reported by Abdullahi-Gero *et al*. (2014). The differences could result from variations in the phytochemical ingredients and concentrations in different parts of the plant and distinctive habitats (Ahmad *et al*., 2016). Also, alkaloids, cardiac glycosides, saponins, flavonoids, phenols, volatile oils and tannins were detected in *A. conyzoides* extract (table 1). The detection of these classes of phytochemicals in the leaves of *A. coznyzoides* collaborates other research reports (Borkataky *et al*., 2013; Odeleye *et al*., 2014; Ajayi *et al*., 2016; Iwuagwu *et al*., 2019). The absence of anthraquinones and steroids in the extract could be due to the inability of methanol to extract certain compounds because of the diverse nature and complex interaction of plant materials (Novy *et al*., 2015).

It has been reported that the use of plants extracts in folkloric medicine for therapeutics purposes could be ascribed to the existence of phytochemicals in plants. For example, saponins have been shown to demonstrate anti-inflammatory effect, immune stimulating activity and antimicrobial properties especially against bacteria (Sahelian, 2014). Tannins, which are polyphenolic compounds and have sufficient hydroxyls have been reported to be active against bacteria especially Gram-negative organisms (Min *et al*., 2008). Alkaloids intercalate into DNA and disrupts its function. This could explain the antimicrobial activity of these extracts (Animashun *et al*., 2023). Flavonoids cause bacterial death by inhibiting DNA or RNA synthesis including possible inhibition of extracellular microbial enzymes (Nyalo *et al*., 2022). These identified groups of phytochemical compounds in the extracts are believed to be a constituent of defense mechanisms in plant, and they can be categorized as protective substances present in this plant and described as "phytoanticipins" and "phytoprotectants" (Egbung *et al*., 2017).

GC-MS analysis of methanolic extract of *A. conyzoides* revealed 17 phytochemical compounds including hexadecenoic acid, methyl ester, 11-octadecanoic acid, methyl stearate, 9-octadecanamide, 1,2-benzenedicarboxylic acid, butyl-2-ethylhexyl ester, silane, trichlorodocosyl-eicosanoic acid, heptadecane,2,3-dimethyl, etc. (table 2). These findings agree with the report of Ahuchaogu *et al*.

(2018). The GC-MC of methanolic leaf extract of *B. verticillata* indicated the existence of a total of 23 phytochemical compounds. The major components were 1.2-15,16-dieoxyhexadecane, 1,2-benzenedicarboxylic acid, butyl 2-ethyhexyl ester, hexadecenoic acid, 2,3-bis (trimethyl islyl) oxyl propyl ester, 9-octadecenamide, 2-ethylbutyric acid, eicosyl ester, 3-tetradecanol, hexadecenoic acid, 2,3-bis [(trimethyl silyl) oxy] peopyl ester, (Z,Z,Z)-8,9-epoxyeicosa 5,11,14-trienoic acid, phenol, 2,4-bis(1,1-dimethylethyl) and 5-Nitrothiophene-2-carboxaldehyde semicarbazone. These phytochemicals were also reported in the investigation conducted by Ogunwande *et al*. (2010). 1,2-benzenedicarboxylic acid and butyl-2-ethylhexyl ester are plasticizer compounds with antimicrobial, antifouling, antioxidant and hyper-cholesterolemic activities (Rubab *et al*., 2020). Hexadecanoic acid (ethyl ester) is a fatty acid ester with nematicidal and pesticidal effect, lubricant, anti-androgenic activity, flavour and possess haemolytic 5-alpha reductase inhibitor properties (Venkalu-Raman *et al*., 2012). 11-octadecanoic acid is a linoleic acid which has antihistaminic, anti-eczemic, and anti-acne properties (Aneesh *et al*., 2013). It is pertinent to identify the possible roles of these constituent compounds in the curative properties attributed to the plant by herbal medical practitioners. Despite the low percentages of some of the detected chemical compounds in this study, scientific reports indicated that each of the compounds had considerable medicinal importance, and the presence of the identified chemical compounds may have an impact on the antibacterial and antioxidant activities of both plants. The chemical compounds identified underscore the veracity of this plant's usefulness in traditional medicine while identified compounds without biological activities found in literature could also contribute individually or synergistically to the pharmacological activities of the extracts (Iwuagwu *et al*., 2019).

Antibiotic susceptibility test conducted according to Kirby Bauer method and in line with CLSI guidelines showed that *K. pneumoniae* was resistant to amoxicillin, ofloxacin and clindamycin but susceptible to the 9 other antibiotics including imipenem and amoxicillin+clavulanic acid but intermediate to gentamycin and septrin with a percentage drug resistance rate of 3(21.43%). *A. baumannii* was resistant to eight (8) antibiotics namely vancomycin, amoxicillin+clavulanic acid, amoxicillin, ofloxacin, ciprofloxacin, erythromycin, rifampicin and clindamycin but sensitive to imipenem, ceftazidime, gentamycin, amikacin and nalidixic acid; it was intermediate to septrin only and had percentage drug resistance rate of 8(53.43%). *E. faecalis* was resistant to five (5) antibiotics including vancomycin, amoxicillin, ciprofloxacin, erythromycin and clindamycin but sensitive to imipenem, amoxicillin+cluvalanic acid, ceftazidime, gentamycin, amikacin ofloxacin, nalidixic acid, rifampicin and septrin while the percentage drug resistance rate was 5(35.71%). The results established the fact that the bacterial isolates exhibited multidrug-resistance (resistance to three or more classes of antibiotics). In cases where bacterial isolates exhibit both MDR and carbapenem resistance, the term "extensive drug resistance" is employed (Sakar *et al*., 2022). However, this phenomenon was not observed in this study because none of the tested organisms was resistant to imipenem, which is a carbapenem.

Members of the family Enterobacteriaceae (*K. pneumoniae* and *E. faecalis*) evaluated in this study showed resistance to amoxicillin (penicillin). Gangoué-Piéboji (2006) reported high (87%) resistance rate of Enterobacteriaceae isolates to amoxicillin in Cameroon. Our finding could be explained by the very frequent use of amoxicillin, especially for self-medication. However, the combination with clavulanic acid restored the potency of amoxicillin against the organisms. Imipenem and amikacin were the most active antimicrobial molecules on the Enterobacteriales with the highest inhibitory zone sizes (table 4). These results are in line with those from Okalla *et al*. (2015) in Cameroon and Abdoulaye *et al*. (2022) in Niger Republic. In addition, *K. pneumoniae* was resistant to ofloxacin (quinolone) and *E. faecalis* was found to be resistant to ciprofloxacin (quinolone). This resistance among enteric bacteria is alarming, and is thought to be the result of the selection pressure created by overuse and misuse of quinolones, especially in the treatment of urinary tract infections and gastroenteritis (Sakar *et al*., 2021). Also, *A. baumannii* demonstrated high resistance to penicillins, quinolones and macrolides in this study but was susceptible to ceftazidime (cephalosporin) contrary to the reports of Pal *et al*. (2017), which stated that *A. baumannii* exhibited high resistance to cephalosporin, whilst Odewale (2016) reported that the organism exhibited 100% resistance to ciprofloxacin and amikacin. This could be as a result of the widespread and simple access to these antibiotics.

In this study, *B. verticillata* leaf extract inhibited bacterial growth of the tested pathogens, producing zone sizes ranging from 7.7 to 17.4mm. This result is in consensus with a past report of Rufa'i *et al*. (2020), which illustrated that the ethanolic flower bud extract of *B. verticillata* exhibited antibacterial activity against *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp. and *Enterobacter* sp. Antibacterial activity of *A. conyzoides* against the organisms revealed lesser zone sizes (9.6 to 14.1mm) with *A. baumannii* having the least zone size (12.6mm) at 100% concentration. This finding concurs with the investigation of Aja *et al*. (2016) who reported antibacterial activity of ethanol leaf-extract of *A. conyzoides* against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp., *Streptococcus* sp. and *Pseudomonas* isolated from wounds. It was also observed that both extracts had higher activity against *E. faecalis*, which is a Gram-positive bacterium than Gram-negative test organism (*K. pneumoniae* and *A. baumannii*). In a report, the methanol, acetone, and chloroform root extracts of *S. singueana* had greater activities on Gram-positive (+ve) bacteria (*Streptococcus pyogenes*, *S. aureus*, and *Streptococcus pneumoniae*) than Gram-negative (−ve) pathogens (*Pseudomonas aeruginosa*, *S. typhi*, *E. coli*, and *Klebsiella pneumoniae*) (Gibremarian *et* 2006). This also concurs with a report by Kareru *et al*. (2007), which showed that the aqueous leaf extracts of *S. singueana* had higher effects on *S. aureus* and *B. subtilis* than it had on *E. coli*. This also agrees with Jibril *et al*. (2021) who demonstrated the broad-spectrum antibacterial effects of methanol and ethyl acetate leaf extracts of *S. singueana*. However, our findings are partly contrary to the reports of Shawa *et al*. (2016), which suggested that aqueous leaf and root extracts of *S. singueana* were inactive on *S. aureus* and *Pseudomonas aeruginosa*. Gram-positive microbes are more susceptible to antibacterial agents because of the characteristic cell wall composition (Breiyeh *et al*., 2020); thus, making them more sensitive to crude plant extracts and bioactive constituents. This could be the possible explanation as to why the studied extracts had higher activities on Gram-positive bacterium.

Conclusion

From the result of this investigation, it can be concluded that *B. verticillata* and *A. conyzoides* present remarkable potential of producing significant plant-derived pharmaceuticals against

multidrug-resistant nosocomial pathogens. However, further research is recommended on the isolation and purification of bioactive molecules, *in vivo* analysis and dosage to determine safety of the phytocompounds.

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Conflict of Interest
None to declare.

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