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Preliminary phytochemical screening and antimicrobial activity of crude extracts of *Bambusa vulgaris* Schrad. Ex J.C. Wendl. (Poaceae) from southwestern Nigeria

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Abstract
Antimicrobial screening of the *n*-hexane, chloroform and ethyl acetate extracts of *B. vulgaris* showed that the crude extracts significantly inhibited the growth of fungi *Aspergillus niger* (minimum inhibitory concentration [MIC] = 1.25 ± 0.80, 2.5 ±0.71 and 2.5 ± 0.71 mg/mL, respectively) and *Verticillium albo-atrum* ([MIC] = 5.0 ± 0.71, 2.5 ± 0.71 and 1.25 ± 0.55 mg/mL) respectively. Antibacterial screening of the plant extracts against Gram-positive *Bacillus cereus* ([MIC] = 1.25 ± 0.23, 2.5 ± 0.55 and 5.0 ± 0.10 mg/mL), *Staphylococcus aureus* ([MIC] = 2.5 ± 0.77, 5.0 ± 0.10 and 1.25 mg/mL), and Gram-negative *Escherichia coli* ([MIC] = 2.5 ± 0.71, 3.5 ± 0.23 and 1.25 ± 0.23) and *Klebsiella pneumoniae* ([MIC] = 3.5 ± 0.23, 2.5 ± 0.10 and 1.25 ± 0.55 mg/mL), respectively, showed that all assayed concentrations significantly inhibited the growth of the microorganisms studied at MIC ≤ 2.5 mg/mL. Therefore the solvent extracts of *B. vulgaris* have antimicrobial activities supporting the ethno medicinal use of aqueous extracts of *B. vulgaris* to treat sexually-transmitted diseases and for wounds.

Keywords: *Bambusa vulgaris* Antimicrobial activity, fungi, bacteria, n-hexane, chloroform, ethyl acetate.

1. Introduction
Bambusa vulgaris Schrad. Ex J.C. Wendl. (Poaceae), a rhizomatous plant commonly known as Golden Bamboo, is widely distributed and grows in tropical and subtropical areas. It is native to Southern China. It is cultivated extensively in many parts of the world especially in wild fields and wet tropics. *B. vulgaris* forms bright green dense tufted culms and grow 10 – 20 m (30 – 70 ft) high and 4 – 10 cm in diameter with thickness ranges 7 – 15 mm [1]. The plant, locally called 'Oparun' (Yoruba), 'Atosi' (Igbo) 'Iko' (Benin) in Nigeria [2], is used for rural construction of houses, huts, boats fences, furniture, musical instruments, basket and decoration, but uses are restricted to the culm and not the leaves [3]. Bamboo leaves have been reported to be used as astringent, ophthalmic solution and febrifuge [2]. Ethnobotanically, *B. vulgaris* is use in traditional medicine for the treatment of several diseases (e.g., measles), as an abortifacient, an appetizer and for managing respiratory diseases. In Nigeria the decoction is taken as a treatment for sexually transmitted diseases (e.g., gonorrhea), diarrhoea, fever, inflammations, ulcers and wounds [4]. The leaves have also been used in India for treatment of various inflammatory conditions [2, 5, 6]. A few members of the genus *Bambusa* species have presented numerous benefits to science and communities [7-10]. Several reports on antimicrobial and antifungal activity of different parts of *Bambusa vulgaris* have appeared from different part of the world [11-14]. In this work, we present the phytochemical screening and antimicrobial activity of crude extracts of *Bambusa vulgaris* growing in southwestern Nigeria.

2. Materials and Methods
2.1 Plant Material
Fresh leaves of *B. vulgaris* were collected in the campus of the Lagos State University (Ojo) during the month of January, 2014. All the chemicals, solvents and reagents used were of AR grade and were purchased from Tunnex Chemicals, Alapere (Lagos). The plant was taxonomically identified and authenticated with voucher number (LUH 6180) at the herbarium of Botany Department, University of Lagos were a voucher specimen is preserved for further use. Prior to crude extract, the plant was air-dried for five days and pulverized. The pulverized plants (400 g) were extracted with hexane, chloroform, ethyl acetate and water successively using Soxhlet extractor for 24 hours to give hexane, chloroform, ethyl acetate and aqueous

extracts. The extracts were collected separately and the solvents evaporated under reduced pressure. The extract yields of the different extracts was determined and represented as percentage with respect to dry weight of the plant material.

2.2 Antifungal and Antibacterial Assays
In this study, the crude extracts were tested for its antimicrobial potential by the agar diffusion method [15]. The fungi (*Aspergillus niger* and *Verticillium albo-atrum*) and Gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) were cultured overnight at 37 ºC in 20 mL of Mueller-Hinton broth (MHB). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of 0.5 McFarland standards at (1.0×10^8) CFU/mL. Petri dishes (90 mm) containing 12 mL of sterilized Mueller-Hinton agar were inoculated with the microbial suspensions. Sterile Whatman No.1 (6 mm diameter) discs papers were individually placed on the surface of the seeded agar plates and 10, 50 and 100 mg/mL of the crude extracts in dimethyl sulfoxide (DMSO) were applied to the filter paper disks. The inoculated plates were incubated at 37 ºC for 24 h. In this study, we used positive control disks comprised of ampicillin and gentamicin (10 mg/disk) for fungi, and bacteria, respectively. Also, dimethyl sulfoxide (DMSO) was used as the negative control. All tests were performed in triplicates. Antimicrobial activity was assessed by measuring the zone of inhibition.

The minimum inhibitory concentrations (MICs) of the crude extracts were determined using 96-well microtiter dilution method as described previously [16]. The bacteria and fungi strains were suspended in Mueller-Hinton broth overnight at 37 ºC and a 1:1 dilution of each strain in fresh MHB were prepared prior to use in the micro dilution assay. Sterile water (100 µL) was pipetted into all wells of the microtiter plate, before transferring of crude extract respectively in DMSO. Serial dilutions were made to obtain concentrations ranging from 100 mg/mL to 10mg/mL. Microorganism cultures (0.1 mL of an approximate inoculum size of 1.0×10^8 CFU/mL) were added to all wells and incubated at 37 ºC for 24 h. After incubation, 40 µL of 0.2 mg/mL, *p*-iodonitrotetrazolium violet (INT) solution was added to each well and incubated at 37 ºC. Plates were examined after about 60-120 min of incubation. Microbial growth is indicated by the presence of a reddish colour which is produced when INT, a dehydrogenase activity detecting reagent, is reduced by metabolically active microorganism to the corresponding intensely colored formazan. Standard antibiotic (ampicillin and gentamicin) and solvent controls (DMSO and hexane) were included in the assay. The growth in each well was compared with that of the growth in the control well. The minimum inhibitory concentrations were detected in comparison with the growth in the control well and delineated as the lowest concentration of the ingredients with > 95% growth inhibition.

2.3 Phytochemical screening
2.3.1 Test for alkaloids
One hundred mg of powdered sample was dissolved in 5 mL of methanol and then filtered. Then 2 mL of filtrate was mixed with 5 mL of 1% aqueous HCl. One milliliter of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive. To the second tube Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids [17].

2.3.2 Liebermann–Burchard test for steroids
Two hundred mg of powder sample was dissolved in 2 mL of acetic acid separately; solutions were cooled followed by the addition of few drops of conc. H_2SO_4. There was no color development from violet to blue or bluish-green was taken as negative test for steroidal ring [17].

2.3.3 Test for saponins
One gram of powdered sample was boiled in 10 mL of distilled water and then filtered. 3 mL of distilled water was added to filtrate and shaken vigorously for about 5 min. There was no formation of foam after shaking a confirmation for the absence of saponins [17].

2.3.4 Shinoda's test for flavonoids
Five hundred milligram of sample was dissolved in 5 mL of ethanol, slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition of few drops of conc. HCl. A pink, orange, or red to purple coloration was taken as a confirmation for the presence of flavonoids [18].

2.3.5 Test for tannins
Five hundred mg of powdered sample was mixed with 10 mL of distilled water and then filtered followed by the addition of few drops of 1% ferric chloride solution. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [18].

2.3.6 Test for phenols
Five hundred mg of extract was mixed with 5 mL of 5% ferric chloride solution. A blue coloration of the solution was taken as a confirmation for the presence of phenol [18].

2.3.7 Test for terpenoids
Five hundred mg of the crude extracts was mixed with 5 mL of chloroform followed by the addition of 3 mL of conc. H_2SO_4. Formation of a brownish colouration at the interface of the mixture was taken as a confirmation for the presence of terpenoid [17].

3. Results and Discussion
The results of different solvent extracts of *B. vulgaris* are shown in Table 1. Crude extracts values were highest in water and *n*-hexane indicating the possibility of considerable amount of polar and nonpolar compounds respectively in the leaves of the plant. The mean values of the phytochemical screening of extracts revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, tannins, alkaloids in the leaf extracts of *B. vulgaris* (Table 2). Steroids and saponins were absent in the crude extracts as shown in the table. The plant extracts were good sources of different classes of bioactive compounds. This indicates that the solvent extracts will be effective in isolating active biological compounds due to their high non-polarity. Terpenoids and flavonoids are typically known for health promoting properties such as antioxidant, anti-inflammatory, antimicrobial and anticancer activities [19]. All the plant extracts exhibited abroad spectrum of antimicrobial activity against some of the tested microorganisms. The extracts exhibited dose dependent increase in activity against the micro-organisms. *n*-hexane extract gave highest inhibition zone (IZ) and minimum inhibition concentration (MIC) (14.0 mm and 1.2.5 mg/mL) against *A. niger*, while ethyl acetate extract exhibited highest

IZ and MIC (16.0 mm and 1.25 mg/mL) against *V. albo-atrum.* The maximum (IZ) and (MIC) against Gram-positive bacteria *B. cereus* and *S. aureus*, were demonstrated in n-hexane (14.0 mm and 1.25 mg/mL) and ethyl acetate (20.0 mm and 1.25 mg/mL) extracts respectively. In addition, *B. vulgaris* extract significantly inhibited the growth of Gram–negative bacteria: *E. coli* and *K. pneumoniae* with highest IZ and MIC (22.0 mm and 1.25 mg/mL) and (18.0 mm and 1.25 mg/mL) for ethyl acetate extract, respectively (Table 3). The result showed that there were no significant differences among different extracts with respect to their antimicrobial activity (MIC > 1.25 mg/mL). Antifungal activity of ethyl acetate extract of *B. vulgaris* leaf showed highest inhibitory activity against the fungus, *V. albo-atrum.* However, *n*-hexane extract showed highest inhibitory efficacy against *A. Niger*. The Overall, efficacy of *B. vulgaris* extracts showed that the ethyl acetate extracts were more effective than the other extracts tested. Recent research on the disc diffusion method of *B. arundinacea* leaf extracts indicated that *n*-hexane showed a good inhibitory effect against *E. coli* (IZ = 22.2 mm; MIC = 3.81 mg/mL), while the chloroform extract demonstrated high inhibitory effect (IZ = 19.0 mm; MIC = 15.5 mg/mL) against *S. aureus*, but was resistant against *E. coli* [20]. This was in agreement with our report. Vijay *et al.* [21] reported on the zone of inhibition of both Gram-positive and Gram-negative bacterial strains using disc diffusion method, that the aqueous extracts of bamboo leaves of *B. arundinacea* showed activity against *E. coli* while the ethanolic extracts demonstrated higher activity against *S. aureus, E. coli, P. aeruginosa* and *B. subtilis* when compared to standard penicillin. Recently, biological activity has been reported for the bamboo species. For example, extracts of leaves and stems of *Phyllostachys* spp. showed promising antibacterial effect [22]. This study demonstrated potent antimicrobial activity for *B. vulgaris,* ethyl acetate extract showing the highest antifungal and anti-bacterial activities and the least by the chloroform extracts. The basis of varying degree of inhibition of the test organisms may be due to the intrinsic tolerance of the microorganism and the nature and combinations of phyto compounds present in the extract as reported by [23]. The control treatment (DMSO) did not show an inhibitory effect on any of the tested fungi and bacteria. Sensitivity of the fungi and bacteria against gentamicin and amoxycillin is presented in Table 3.

Several Gram-negative bacteria exhibited high level on intrinsic resistance against antimicrobial and antibiotics, due to a very restrictive outer membrane barrier which is highly resistant even to synthetic drugs [24].

Table 1: Different extracts of *B. vulgaris*

Solvent	Extractive values (%)
Chloroform	4.5 ± 0.55
n-hexane	6.6 ± 0.23
Ethyl acetate	3.8 ± 0.20
Water	7.0 ± 0.44

Table 2: Phytochemical analysis of the crude extract of *B. vulgaris*

Constituents	Chloroform	*n*-hexane	Ethyl acetate
Alkaloids	+	+	+
Steroids	-	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Phenols	-	-	+
Terpenoids	+	+	+
Saponins	-	-	-

Key+ = present; - = absent

Table 3: Anti-microbial activity of crude extracts of *B. vulgaris* against different fungi and bacteria (zone of inhibition and Minimum Inhibition Concentration)

Micro organism			Zone of inhibition (mm)				
		Conc. (mg/mL)	CE	HE	EE	AM	GE
Fungi	*A. niger*	10.0	9.0 ± 0.23	8.0 ± 0.77	10.0 ± 0.58	16.0 ± 0.55	19.5 ± 0.23
		50.0	11.0 ± 0.33	12.0 ± 0.88	13.0 ± 0.77	22.0 ± 0.10	23.0 ± 0.10
		100.0	15.0 ± 0.33	14.0 ± 0.55	16.0 ± 0.23	26.0 ± 0.55	25.0 ± 0.88
		MIC	2.5 ± 0.71	1.25 ± 0.80	2.5 ± 0.71	0.31 ± 0.10	0.31 ± 0.10
	V. albo-atrum	10.0	8.0 ± 0.10	10.0 ± 0.33	11.0 ± 0.55	14.0 ± 0.55	15.0 ± 0.10
		50.0	10.0 ± 0.80	11.0 ± 0.10	14.0 ± 0.55	16.0 ± 0.55	19.0 ± 0.40
		100.0	12.0 ± 0.88	14.0 ± 0.55	16.0 ± 0.10	20.0 ± 0.33	23.0 ± 0.10
		MIC	2.5 ± 0.33	5.0 ± 0.71	1.25 ± 0.55	1.25 ± 0.77	2.5 ± 0.33
Bacteria	*S. aureus*	10.0	10.0 ± 0.23	11.0 ± 0.88	12.0 ± 0.10	14.0 ± 0.55	12.0 ± 0.88
		50.0	12.0 ± 0.88	12.0 ± 0.90	14.0 ± 0.66	19.5 ± 0.23	20.0 ± 0.33
		100.0	14.0 ± 0.66	15.0 ± 0.10	20.0 ± 0.33	25.0 ± 0.88	26.0 ± 0.55
		MIC	5.00 ±0.10	2.5 ± 0.77	1.25 ± 0.33	0.63 ± 0.33	0.63 ± 0.55
	B. cereus	10.0	7.0 ± 0.88	8.0 ± 0.33	12.0 ± 0.90	14.0 ± 0.66	13.0 ± 1.10
		50.0	9.0 ± 0.33	10.0 ± 0.58	14.0 ± 0.20	16.0 ± 0.10	15.0 ± 0.10
		100.0	12.0 ± 0.88	14.0 ± 0.66	10.0 ± 0. 33	23.0 ± 0.10	21.0 ± 0.23
		MIC	2.5 ± 0.55	1.25 ± 0.23	5.0 ± 0.10	0.63 ± 0.23	0.63 ± 0.23
	E. coli	10.0	7.0 ± 0.88	8.0 ± 0.77	9.0 ± 0.55	11.0 ± 0.33	14.0 ± 0.55
		50.0	9.0 ± 0.33	10.0 ± 0.80	11.0 ± 0.55	15.0 ± 0.10	16.0 ± 0.23
		100.0	14.0 ± 0.55	19.0 ± 0.40	22.0 ± 0.20	25.0 ± 0.88	26.0 ± 0.55
		MIC	3.5 ± 0.23	2.5 ± 0.71	1.25 ± 0.23	0.31 ± 0.10	0.63 ± 0.23
	K. pneumonia	10.0	6.0 ± 0.10	7.0 ± 0.88	8.0 ± 0.33	13.0 ± 0.10	20.0 ± 0.10
		50.0	8.0 ± 0.20	9.0 ± 0.33	10.0 ± 0.88	21.0 ± 0.10	23. 0± 0.10
		100.0	12.0 ± 0.88	12.0 ± 0.88	18.0 ± 0.33	24.0± 0.33	26.0 ± 0.23
		MIC	2.5 ± 0.10	3.5 ± 0.23	1.25 ± 0.55	0.31± 0.33	0.31± 0.23

MIC values are given as (mg/mL) [CE – chloroform, HE – *n*-hexane, EE – ethylacetate AM – ampicillin and GE - Gentamicin].

4. Conclusions

The phytochemical constituents present in *B. vulgaris*, namely alkaloids, tannins, flavonoids and terpenoids, have demonstrated promise for the expansion of modern chemotherapies against microbial infections. Several studies have assessed these metabolites and have been reported by many researchers to be bioactive and have been confirmed by previous works to have medicinal as well as physiological properties and therefore could be said to be responsible for the activity of *B. vulgaris* in treatment of different ailments [25, 26]. Many groups of flavonoids have been used to treat human diseases [27]. Many phenolic terpenoids as well as carotenoids have been shown to enhance oxidation resistance [28]. The results of this study confirm some scientific credence to the traditional use of the medicinal plants for treatment of several infectious diseases and wounds. In addition, preliminary phytochemical profile has revealed that the phytochemical composition varies with the solvent used. Further studies are needed to establish the mechanism of action and isolation of phytochemical composition responsible for the concerned activity.

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