Archives of Ecotoxicology, Vol. 5, No. 2, pp. 45-51, 2023

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Archives of Ecotoxicology

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Phytochemical Compounds and Antimicrobial Activity of Extracts of Bamboo Against *Staphylococcus aureus* and *Escherichia coli*

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Article info
Received 5 July 2023
Revised 1 November 2023
Accepted 7 November 2023
Published online 10 November 2023

Regular article

Keywords:
Bambusa vulgaris,
Thin Layer chromatography,
Candida albicans,
Staphylococcus aureus,
Escherichia coli,
phytochemicals

Abstract

Plants contain bioactive compounds which can be used to develop novel antibiotics. There is need to search for new antimicrobial compounds to address the problem of microbial resistance against antibiotics. *Bambusa vulgaris* plants have been used in traditional medicine to manage respiratory diseases, cardiovascular diseases and gonorrhea. Little research has been done on phytochemical and antimicrobial activity of *Bambusa vulgaris*. The purpose of this study was to screen phytochemical compounds present in the roots and leaves extracts and to test antimicrobial activity of the extracts of *Bambusa vulgaris* against *Staphylococcus aureus* and *Escherichia coli*. The plants were collected from Maseno University Botanic Garden. Roots and leaves were dried separately, ground into fine powder and extracted using Soxhlet method. Phytochemicals: flavonoids, saponins, terpenoids, tannins, alkaloids and glycosides were tested. Thin layer chromatography plate was coated with silica gel. n-Hexane and Ethyl acetate were used as solvents in the ratio 2:3 respectively. Different concentrations of plant extracts were prepared; 25%, 50%, 75%, and100% (stock extract) and Positive control (Amoxicillin). The bacteria were inoculated into the nutrient agar. Antimicrobial activity of acetone extracts of leaves and roots were carried out by disc diffusion method. Diameter of inhibition zones was measured using a ruler. The leaves extracts were found to contain saponins, flavonoids, glycosides tannins and terpernoids. The root extracts contained saponins, glycosides and tannins. TLC revealed that there were 7 spots in the leaves and 4 spots in roots extract. Increase in concentration of the leaves and roots extracts of *Bambusa vulgaris* significantly inhibited the growth of *Staphylococcus aureus* and *Escherichia coli.* Leaves extracts showed a higher inhibition activity compared to roots. The growth inhibition may be attributed to the presence of the various phytocompounds. The study has shown that *Bambusa vulgaris* leaf methanol extract has antimicrobial activity against gram positive *(Staphylococcus aureus)* and gram negative *(Escherichia coli) bacteria* and hence can be used as source of bioactive agents to control these pathogens.

1. Introduction

Plants have a great potential to produce new drugs for human benefit (Ambika and Rajagopal, 2017a; Sharma et al., 2020). About 75% - 80% of the world's population in the developing countries relies on herbal medicines for primary healthcare **(Mohankumar *et al.*, 2017)**. Traditional medicine is an important but often underestimated part of health services **(Kigen *et al.*, 2017)**. Phytochemicals are biologically active biological compounds occurring in plants **(Saxena *et al.*, 2013)**. Many herbaceous and medicinal plants have phytochemicals such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins and lignans and vitamin C **(Hussan *et al.*, 2011; Coffie *et al.*, 2014)**. Phytochemicals like alkaloids, phenolics, terpenoids and tannins have a potential to prevent diseases and act as antimicrobial, anti-inflammatory, anti-oxidant, anti-cancerous, detoxifying agent, immunity potentiating agent and neuropharmacological agent **(Shirsat *et al.*, 2016)**. Phytochemicals are classified as anticancer, antioxidants, antibacterial, detoxifying agents, non starch polysaccharide and others **(Saxena *et al.*, 2013)**. Different plants and plant parts such as the leaf, root, flower, fruit, peel, bark, seed, stem, rhizome, etc. can be used for therapeutic purposes because they have high amounts of bioactive compounds **(Ifesan *et al.*, 2013)**. Medicinal plants are known to harbor potential endophytic microbes, due to their bioactive compounds **(Egamberdieva *et al.*, 2017)**. The antimicrobial property of medicinal plants may differ depending on the forms of added plants, such as fresh, dried, or extracted forms. Production of phytochemicals in plants is depended on many pre- and post-harvest factors including farming practices, environmental factors (microclimate, location, growing season, soil type and nutrients), plant maturity, post-harvest storage and processing and genetics **(Li *et al.*, 2012)**. The antimicrobial

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https://doi.org/10.36547/ae.2023.5.2.45-51

properties of plants have been investigated by a number of investigators worldwide **(Nascimento *et al*., 2000)**. Evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy can lead to the discovery of new and cost-effective drugs.

Bamboo is an extremely diverse plant which easily adopts different climatic and soil conditions **(Loboviko *et al.,* 2007)**. The most ideal temperature range for most bamboo growing is 15-20^0 C, with annual rainfall of 1000-1500mm **(Karanja, 2017)**. Bamboo shoots are reported to have anticancer, antibacterial, antiviral activity **(Ambika and Raijagopal, 2017b).** The roots have been used to check blood flow and restrict secretions of body fluids and also in treatment of rabies **(Shukla *et al.,* 2011*)*.** Chloroform extract of the leaves has been used against *Mycobacterium tuberculosis*. Bamboo leaves have been used in traditional Chinese medicine for treating fever and detoxification **(Soni *et al.,* 2013)**. Bamboo is generally considered cooling, calming, and phlegm resolving, and has been incorporated in many traditional formulas to treat lung and stomach heat and febrile disease **(Panee, 2015)**. Bamboo leaves in Asian traditional medicine are used for the treatment of hypertension, arteriosclerosis, cardiovascular disease, and certain forms of cancer **(Katarzyna *et al.,* 2019**; **Soni *et al*., 2021)**. Medicinal properties of bamboo leaves have shown antioxidant, anticancer, and antibiotic properties **(Tanaka *et al.*, 2012)**. Extracts and compounds from the leaves of *Sasa senanensis* (Franch. & Sav.) Rehder, *Phyllostachys nigra* (Lodd.) Munro, and *Phyllostachys edulis* (Carrière) J.Houz have been studied, although a few studies have investigated on the leaves of other bamboo species **(Panee, 2015; Karanja, 2017)**. The activity of bamboo extracts is different in each species **(Rahayu and Sukmawati, 2015)**. *Escherichia coli* is a gram-negative bacteria commonly found in the intestines of humans and animals, and *Staphylococcus aureus* is a gram-positive bacteria frequently found in the human respiratory tracts and skin **(Lemos and Abranches, 2013)**. Gram-positive bacteria have cell walls composed of thick layers of peptidoglycan while Gram-negative bacteria cell walls have a thin layer of peptidoglycan. The difference in composition and structure of cell walls in gram negative and gram-positive bacteria can affect the antibacterial activity of a phytochemical compound. Diseases caused by microorganisms are a major problem to animals and humans. The increase to antibiotics resistance has led to the need to search for new antimicrobial agents. Antibiotics are also increasingly becoming expensive. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action **(Priscilla *et al*., 2014)**. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery. This study was designed to investigate the phytocompounds and antimicrobial activity of *Bambusa vulgaris* root and leaf methanol extracts against *staphylococcus aureus and Escherichia coli*. It was hypothesized that *Bambusa vulgaris* has many phytochemical compounds and the crude leaf and root methanol extracts inhibits the growth *Staphylococcus aureus* and *E. coli.*

2. Materials and methods

2.1 Identification and Collection of plant parts

The leaves and the root of *Bambusa vulgaris* which were used in this study were collected from Maseno University Botanic Garden. The plant specimens were cleaned off soil or dust by shaking and packed in carrier bags which were then transported to the Department of Botany, Maseno University for taxonomical identification and authentication. The voucher specimens were submitted to the same department.

2.2 Preparation of plant extracts

The leaves and roots were air-dried separately under the shade for two weeks and cut into small pieces. They were then ground using a heavy duty laboratory mill into a fine powder, and stored in airtight plastic containers at room temperature (25-30^{o}C) for extractions. This prevented absorption of moisture from the atmosphere. One thousands grams of the powdered roots and leaves were weighed using a top loading balance (Denver instrument) and were transferred into 2 five liter conical flasks (Pyrex) according to the procedure of **McCloud, (2010)**. Then 2.5 liters of 99% methanol was added to cover the plant material under fume hood and left to soak in the solvent at room temperature for 5 days with shaking on rotary shaker (Digisystem laboratory Instrument incl) with a speed of 20 revolutions per minute. Extracts were filtered through No. 1 Whatman filter paper on a Buchner funnel under vacuum pump (Vacuubrand GMBH). The extracts were concentrated in a rotary evaporator pump (Eyela SB-1000), and the dry extracts were stored at 4˚C before performing the biological activities.

2.3 Preparation of Antibiotics

Amoxicillin in capsules (AMBIMOX) were used. Stock solution (1 mg/mL) of the antibiotic was prepared by weighing, and dissolving 5 mg of amoxicillin powder into a 5 mL sterile H_2O. The amoxicillin solution was further homogenized using a vortex mixer. It was then filter sterilized and stored in a refrigerator.

2.4 Phytochemical compounds screening

The phytochemical screening of methanol extract were performed by qualitative chemical tests. The tests were performed for the presence of saponins, flavonoids, glycosides, tannins, alkaloids and terpenoids by adopting standard protocols **(Trease *et al*., 2002; Congesta *et al*., 2005, Antara and Batra, 2012; Minakshi *et al*., 2016)**.

2.4.1 Saponins

Each of the extracts was weighed to 0.5mg and dissolved in 1 ml of methanol and filtered. Distilled water was added and shaking done for a few minutes. If there was persistence frothing then it indicated the presence of saponins.

2.4.2 Flavonoids

Each of the extracts was weighed to 0.5mg and dissolved in 1 ml of ethanol and filtered. 2ml of 1% HCl and magnesium ribbon was added to the filtrate. If there was the formation of a pink or red colour it indicated the presence flavonoids.

2.4.3 Glycosides

To 0.5g each of plant extract in a test tube, was mixed with 2ml of glacial acetic acid containing 1 drop of $FeCl_3$.1ml of concentrated sulphuric acid was then added to the mixture. A brown ring formation indicated the presence of glycosides.

2.4.4 Tannins

Each of the extracts was weighed to 0.5mg and dissolved in 1 ml of distilled water. Filtration was carried out after 2ml of $FeCl_3$ was added. If there was the presence of a blue or black precipitate then it indicated the presence of tannins.

2.4.5 Alkaloids

Each of the extracts was weighed to 0.5mg and dissolved in 1ml of methanol and filtered. 1% HCL was added to the filtrate and the solution heated. Mayor`s reagent was added dropwise and if there was the formation of any colored precipitate it indicated the presence of alkaloids.

2.4.6 Terpenoids

To 0.5g of crude extract in a test tube, 2ml of chloroform was added followed by 3ml of 0.1M Sulphuric acid. Formation of a reddish brown colouration at the interface of the mixture was taken as a confirmation for the presence of terpenoid.

2.5 Thin layer chramatography (TLC)

TLC is a rapid and economical procedure for the determination of the main active principles of medicinal plants. Thin layer chromatography was carried on leaf and root extracts of *Bambusa vulgaris* according to **Musa *et al.* (2017)**. Using an aluminium TLC plate whose surface was coated with silica gel, the TLC plate was cut into sizes of 10cm by 5cm and a mark was made using a pencil. The mark was about 1.5cm from the lower edge of the end of the plate which was to represent the starting point during elution. The dissolved extracts were then spotted on the line creating a distance of about 0.5 -1.0cm between each spot. The distance between the spots was mainly to prevent the spots from mixing or overlapping during separation. Solvent system was prepared in the development chamber by mixing n-hexane and ethyl acetate in the ratio of 2:3 respectively. The spotted plate was placed in the development chamber such that the solvent system did not touch the spot. The solvent moved through the plate by capillary action. The compounds present in the extract were then separated depending on their solubility into the solvent and the adsorption capacity of the compounds on the silica adsorbent and appeared as spots on the plate. The spots were visualized using a UV lamp (Uvitec model) to enhance visualization at different wavelengths of 365nm and 254nm. The spots were circled using a pencil and the distances travelled were measured using a ruler. The solvent front distances were also measured and recorded. The retention factor (R_f) was calculated using the formula proposed by **Musa *et al.* (2017)**:

R_f = *Distance travelled by solute /Distance travelled by solvent front*

The retention factor **(Rf)** value essentially describes the distance travelled by the individual component **(Rao and Shil, 2019; Musyimi *et al.*, 2022**).

2.6 Culture media

Staphylococcus aureus and Escherichia coli were obtained from the department of Botany Maseno University, Kenya. They were maintained in petri dishes containing nutrient agar media. Petri dishes were incubated overnight to ensure sterility before use.

2.7 Antimicrobial activity

Plant extracts were prepared by diluting the stock solution (100%) in water to make concentrations of 0%, 25%, 50%, and 75%. The test organisms (100µl) were swabbed aseptically into a solidified nutrient agar by streaking the cotton swab over the entire surface of the agar plate 3 times. Circular discs of 6mm diameter each were cut from laboratory grade filter using a paper punch and each was dipped in a known concentration of plant extracts for 3 minutes according to **Musyimi *et al.* (2008)**. The agar plates with the disks were allowed to stand for an hour at room temperature. The plates were then incubated at 37°C. After 16 to 24 hours, the clearing around the paper disc (zone of inhibition) was then observed. The plates were the inverted and the diameter of each inhibition zone was then measured in millimeters. The diameter of the disk paper was not included in the calculation of the inhibition zone. The positive control used was the amoxicillin antibiotic because amoxicillin is a broad-spectrum antibiotic that works by preventing the synthesis of bacterial cell walls.

2.8 Data Analysis

The data obtained from the study was subjected to analysis of variance (ANOVA) using SAS statistical package. Means were separated and compared at ($p<0.05$) for any significant difference between treatments and the microorganisms.

3. Results

3.1 Phytocompounds analysis

The leaf and root extracts were screened for the presence of active secondary metabolites. The leaves extracts were found to contain saponins, flavonoids, glycosides, tannins and terpernoids but lacked alkaloids (table 1). The root extract contained saponins, glycosides and tannins but flavonoids, alkaloids and terpenoids were absent.

Table 1 Results of preliminary qualitative phytochemical analysis of leaves and roots extracts of *Bambusa vulgaris*

Secondary metabolites	**Leaf extract**	**Root extract**
Saponins	**+**	**+**
Flavonoids	**+**	**-**
Glycosides	**+**	**+**
Tannins	**+**	**+**
Alkaloids	**-**	**-**
Terpenoids	**+**	**-**

Key: + Present - absent.

3.2 Thin layer chromatography

Elution of the column with the n-hexane and ethyl acetate in the ratio 2:3 led to isolation of 7 spots of uncharacterized spots in leaves and 4 spots in the roots (Table 2). The leaves extract had the highest number of spots compared to the roots extract.

3.3 Antimicrobial activity of *Bambusa vulgaris* leaves and roots extracts

Results on antimicrobial activity revealed that leaves extract greatly inhibited the growth of the microorganisms as compared to the roots extract (Tables 3, 4 and 5). Inhibition zones diameters increased significantly with extract concentration. This showed that the higher the concentration of the extract the higher the activity against the test microorganism. There was a significant difference between *S. aureus* and *E. coli*. There was a significant difference ($p<0.05$) in growth inhibition between the plant extracts and the positive control (amoxyllin). The positive control showed a greater diameter of inhibition compared to the different plant extracts concentrations. There was a significant difference ($p<0.05$) in growth inhibition between the leaves and roots on the three test microorganisms.

Table 2 *The* R_f values of active compounds isolated from the methanol leaf and root extracts of *Bambusa vulgaris* using thin layer chromatography (TLC)

Plant part extracts	**Visualization at 365nm**	Number of spots	R_f values
Roots	UV Lamp	1	0.21
	UV Lamp	2	0.55
	UV Lamp	3	0.70
	UV Lamp	4	0.95
Leaves	UV Lamp	1	0.09
	UV Lamp	2	0.19
	UV Lamp	3	0.57
	UV Lamp	4	0.68
	UV Lamp	5	0.88
	UV Lamp	6	0.92
	UV Lamp	7	0.96

Table 3 Antimicrobial activity of *Bambusa vulgaris* leaves and roots extracts at different concentrations

		Growth inhibition diameter (mm)
Microbes	*Staphylococcus aureus*	1.57[b]
	Escherichia coli	3.39[a]
	LSD	0.22
Plant extract concentrations	0% (negative control)	0.00[d]
	25%	1.06[c]
	50%	1.14[c]
	75%	1.50[b]
	100%	1.58[b]
	Positive control (amoxyllin)	7.21[a]
	LSD	0.31
Plant parts	Roots	1.37[b]
	Leaves	2.79[a]
	LSD	0.18

Means with the same letter are not significantly different. Data presented are means of three replicates.

Table 4 Growth inhibition zones of the microbes at each concentration of the leaves extract

Microbes	**Concentration of plant extracts**	**Inhibition zone (mm)**
Staphyllococcus aureus	0% distilled water (negative control)	0.00±0.00
	25%	1.17±0.08
	50%	2.17±0.08
	75%	2.17±0.08
	100%	2.67±0.08
	Positive control (amoxyllin)	5.01±0.10
Escherichia coli	0% distilled water (negative control)	0.00±0.00
	25%	1.67±0.33
	50%	1.67±0.33
	75%	2.33±0.58
	100%	6.33±1.08
	Positive control (amoxyllin)	13.20±0.73

Data presented are means of three replicates ± S.E.

Table 5 Growth inhibition zones of microbes at each concentration of the roots extract

Microbe	Concentration of plant extract	Inhibition zone (mm)
Staphyllococcus aureus	0% distilled water (negative control)	0.00±0.00
	25%	0.00±0.00
	50%	0.00±0.00
	75%	0.17±0.08
	100%	0.50±0.25
	Positive control (amoxyllin)	5.01±0.10
Escherichia coli	0% distilled water (negative control)	0.00±0.00
	25%	0.00±0.00
	50%	0.17±0.08
	75%	0.33±0.08
	100%	1.83±0.08
	Positive control (amoxyllin)	13.20±0.73

Data presented are means of three replicates ± S.E.

4. Discussion

The medicinal value of plants lies in some chemical substances that produce definite physiological action on the human body **(Musyimi *et al.,* 2008).** There is need to search for effective compounds which would enable protection against pathogens. Phytochemical screening of leaves and roots methanol extract of *B. vulgaris* (table 1) revealed the presence of alkaloids, tannins, phenolics, glycosides, saponins, flavonoids and terponoids. The results are in agreement with **Coffie *et al*. (2014); Owolabi and Lajide, (2015); Yakubu and Bukoye, (2009); and Wani *et al*. (2019)**. Phytochemical analysis results were also in agreement with Kebede *et al. (*2021) who studied various selected medicinal plants. Saponins and phenols were reported to be absent in the leaves crude extracts *B. vulgaris* by **Owolabi and Lajide, (2015**) using chloroform and n- hexane solvents in disagreement with the current results. The absence of alkaloids in the leaves and alkaloids and terpenoids in the roots crude extracts was possibly due to the organic solvent used in the test. It should be noted that different organic solvents have distinctive capabilities in extracting biochemical constituents from a sample **(Iwalokun *et al*., 2007)**. Screening of medicinal plants secondary metabolites assists in the detection of bioactive compounds **(Aziz, 2015)**. Thin layer chromatography gives semi quantitative information about the main active constituents present in the plant extract **(Banu and Nagarajan, 2014; Musyimi *et al*., 2022)**. Compounds with high Rf values in less polar solvent systems have low polarity while those with low Rf values have high polarities. In this study thin layer chromatography confirmed the presence of potentially active metabolites. The extracts displayed antimicrobial potential against the pathogenic microorganisms tested. Flavonoids are very important secondary metabolites reported to possess antibacterial, antiviral, anti-inflammatory, enzyme inhibition and antioxidant properties **(Cushnie and Lamb, 2005)**. Phenols are known to contain high level of antioxidant activities due to the presence of hydroxyl groups which can eliminate free radicals. Glycosides support the defense mechanism against microbes, insects, and herbivores. Saponins exhibit significant hypocholesterol, hypertensive, and cardiac depressant properties **(Sharma *et al*., 2017)**. Phytochemicals such as flavonoids and glycosides are known for antioxidant activity **(Duraiswamy *et al*., 2018)**. The antibacterial effectiveness of tannins is explained by their ability to pass through the bacterial cell wall up to the internal membrane, interference with the metabolism of the cell, and as a result their destruction. Tannins are multidentate ligands which may bind to proteins, mainly by hydrophobic interactions and hydrogen bonds inhibiting bacteria metabolism. **Dabbaghi *et al*. (2009)** have reported the tannic acid activity against *Staphylococcus aureus* and *Escherichia coli* which dependend on the phenolic hydroxyl groups content. Flavonoids produce significant anti-inflammatory and antimicrobial action which is due to their capacity in complexing extracellular and soluble proteins such as structures from bacterial cell walls. Alkaloids are toxic to microbes and they also possess antifungal and anti-inflammatory and antihypertensive agents **(Sofowora, 1993; Okwu, D.E. and M.E. Okwu, 2004)**.

There was a significant difference in growth inhibition between *E. coli* and S*taphylococcus aureus* (tables 3). Results clearly indicated that *E. coli* was more sensitive to the inhibitory effects of the plant extracts than *S. aureus* at 100% extract concentration (tables 4 and 5). Inhibition zones diameters also increased with extract concentration. Similarly, **Ambika and Rajagopal (2017b)** showed that the acetone extract of *B. vulgaris* shoots, exhibit antibacterial activity against five strains of bacteria. In addition, n-hexane and ethyl acetate extracts of *B. vulgaris* were found to demonstrate antibacterial activity against gram-positive bacteria *Bacillus cereus* and *S. aureus* **(Owolabi and Lajide, 2015)**. The results may reveal that the type and the level of biological activity exhibited by plant materials may depend on the plant part used, geographical source of the material, moisture content, method of drying method, type of solvent used in extraction and storage conditions. Active ingredients of medicinal plants may be poorly or totally not extracted if the right solvent is not used.

Amoxicillin was used as a reference standard for comparing the antibacterial potential of the methanolic root and leaf extract of Bamboo (*Bambusa vulgaris*). Amoxyllin antibiotics (positive control) showed a greater diameter of growth inhibition compared to the different plant extract concentrations. There was also a significant difference in growth inhibition between the plant parts extracts on the test organisms. The antimicrobial properties of *Bambusa vulgaris* plant could be due to the phytocompounds present in plant extracts **(Musyimi *et al*., 2008)**. Differential sensitivity of microbes to the extracts may be explained by the cell wall composition of the gram positive and gram-negative bacteria. The cell wall of gram negative bacteria contains the outer membrane and lipid bilayer embedded with proteins and porins. Apart from difference in phytochemical composition, the plant part used, geographical location, season, and climatic conditions also may affect antimicrobial activity of plants **(Barral-Martinez *et al.,* 2022)**. The study on antimicrobial activity is in agreement with **Sonia *et al. (*2021)** who used *Dendrocalamus hamiltonii* Bamboo leaves and stems extracts. The present study suggests that *Bambusa vulgaris* extracts presumably possess compound(s) with antimicrobial properties against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. The gram positive bacterium has a relatively thick layer of peptidoglycan sheets of interconnected glycan chains made up of polymer and thus sensitive to most extracts. The gram negative bacteria have lipoproteins and lipopolysaccharides that form a barrier to hydrophobic compounds. Gram-negative bacteria are considered to be more resistant due to their outer membrane which acting as a barrier to many environmental substances including antibiotics. The results have revealed that the leaves and roots of *Bambusa vulgaris* contain several phytochemicals with broad antimicrobial activities. However, it should be noted

that the methods of extraction and agroecological zone where plant material samples were collected may influence on the effectiveness of the extracts on the test organisms.

5. Conclusion

The study has revealed that the leaves of *Bambusa vulgaris* are rich in saponins, flavonoids, glycosides, tannins and terpenoids, and the roots are rich in saponins, glycosides and tannins. Thin layer chromatography has revealed that the leaves extracts has more active compounds compared to the roots methanol extract. The study has shown that *Bambusa vulgaris* leaf methanol extract has higher antimicrobial activity against *E. coli*, and *S. aureus* compared to roots extracts. The results provide partial scientific validation for using *Bambusa vulgaris* against infectious diseases in different communities. Due to limited research funding, we were unable to purify and identify the specific bioactive compounds in the methanol extracts of the plants. We recommend future researchers to use high-pressure liquid chromatography (HPLC) along with nuclear magnetic resonance (NMR) to identify the active ingredients. It is also recommended that further analysis on the methanol extracts of *Bambusa vulgaris* be conducted to identify which of the isolates exhibited the identified inhibitory activity.

Declaration of interest

The authors declare no conflicts of interest.

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