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Antibacterial Activity of Dichloromethane, Hexane, Ethanol and Methanol Extracts of *Asparagus setaceous* Kunth and *Caesalpinia volkensii* Harm

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ABSTRACT

Two medicinal plants extracts traditionally used in Kenya mainly for the management of infectious conditions were chosen and screened for their antibacterial activity against gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus faecalis* and gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Antibacterial activity was tested using disc diffusion method. Dichloromethane, hexane, methanol and ethanol extracts of *A. setaceous* and *C. volkensii* showed activity against the bacteria. These organic extracts showed minimum inhibitory concentration (mic) against the tested bacteria with mic ranging from 6.25-50mg/ml.

INTRODUCTION

Nature has been a source of medicinal agents for thousand of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. Plants have a great potential for producing new drugs of benefit to mankind [2]. During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance bacteria is no longer matched by expansions in the arsenal of agents [3]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as soil microorganism, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds [4]. There is therefore urgent need to screen a number of medicinal plants for promising biological activity. There exist several reports on the antimicrobial activity of different herbal extracts in different region of the world [5,6, 7, 8]. Because of the side effects and the resistance that pathogenic microorganism have built against antibiotics, much attention is now being paid to extracts and biologically active compound isolated from plant species used in traditional medicine. Plant based antimicrobials represent a vast untapped source of medicine and further exploration and exploitation of plant antimicrobials in treatment of infectious disease is needed. Two medicinal plants used in the traditional setup in Kenya and belonging to two different families were screened for their antimicrobial properties. *Asparagus setaceous* belongs to the family Liliaceae while *Caesalpinia volkensii* belongs to Caesalpinaceae. *A. setaceous* is a monocotyledonous plant that is distributed in most parts of Kenya while *C. volkensii* on the other hand is a dicotyledonous plant that is mainly distributed in five of the eight provinces of Kenya namely Rift valley, Central, Nairobi, Western and Coast [9]. These two plants have reportedly been used in the treatment of inflammatory diseases, bronchitis, pneumonia, syphilis, gonorrhea, malaria and helminthic infections [10,11,12] and all parts of the plants have reportedly been used.

A survey of the literature reveals that these two plants have barely received scientific attention if any and there is lack of concrete scientific data on their antimicrobial activity in spite of their widespread use in the traditional medicine practices of most communities in Kenya. The purpose of this study therefore was to screen the organic solvent extracts of these medicinal plans that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases.

MATERIALS AND METHODS

Experimental Animals

Male and female albino rats weighing between 140-210g used in the study were obtained from the animal house in the Department of Zoology, JKUAT. They were housed five per cage, were maintained in animal room under a 12:12-h light-dark cycle at a temperature of 25 ° C and fed on rat pellet and tap water *ad libitum*.

Materials

Methanol, ethanol, dichloromethane, hexane were purchased from Fisher Scientific, UK, Ltd (Bishop Meadow Road, Loughborough, Leicestershire, LE 11 5RG, UK). Mueller Hinton agar were purchased from Biotech laboratories Ltd. UK, while dimethylsulfoxide (DMSO) was purchased from Sigma (Poole, Dorset, England).

Plant materials and their collection

Aerial part and root of *Asparagus setaceous* and the leaf, stem and root of *Caesalpinia volkensii* were colleted from Gatundu [1°3'0"S: 36°54'0"E] located in central province of Kenya. Gatundu is approximately 40km north of Nairobi. The plants were identified in the herbarium, Department of Botany JKUAT, where voucher specimens were deposited. The plant materials were dried under shade at temperature below 30° and pulverized in a hammer mill fitted with a sieve of 0.5mm pore.

Preparation of organic extracts

The grounded plant material was extracted twice with organic solvents, hexane, dichloromethane, methanol and 90% ethanol at room temperature 100g of plant powder were extracted by mixing with 300ml of the extracting solvent. The slurry of solvent and plant powder was stirred and left to stand for 48 hours, after which the supernatant was decanted. The residue was then extracted one more time before being discarded. The decanted supernatants were filtered through Whatman ®GF/C glass microfibre filter paper and the filtrate concentrated under vacuum at 40°C in Buchii rotary evaporator and dried in a freeze drier. The dry extracts were weighed and kept desiccated at 4°C until required.

Antibacterial screening of plant extracts crude extracts on selected pathogens

Antibacterial activity of the plant extracts of *A. setaceous* and *C. volkensii* were tested by the disc-diffusion method. The minimal inhibition concentrations (MIC) of the extracts were also determined. Five bacterial strains, *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis* and the fungus C*andida albicans* were used in the investigation. These micro organisms except *B. subtilis* are medically important. The organisms were obtained from a culture collection maintained in the Department of Botany, JKUAT. The bacteria were tested for purity by culturing on nutrient agar and maintained on nutrient agar slants.

Preparation of inoculums

Stock cultures were maintained on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria to reactivate them by culturing overnight at 37◦ C. Cultures were diluted with fresh MHB and compared with McFarland standard to achieve values corresponding to $2x10^6$ colony forming unit for bacteria.

Antibacterial activity of the extracts

Antibacterial activity of the plant extracts was tested for by the disk diffusion method as described by [8]. Five strains of bacteria were used, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa (*ATCC 27853), *Streptococcus faecalis Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (ATCC 6633). Filter paper discs (Whatman No. 1) 6 mm diameter were impregnated with crude extracts (25mg/disc) or standard drugs. Disks dipped into dimethylsulfoxide served as negative controls. All the bacteria mentioned above were incubated at 30°C for 24h by inoculation into nutrient broth. Sterilized petri dishes were inoculated with 0.01 ml of one of the above culture media (10^5 10^6 bacteria per ml). Mueller Hinton agar sterilized in a flask and cooled to 45 50° C was distributed by pipette (15ml) into each inoculated petri dish and swirled to distribute the medium homogenously. Disks injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri-dishes were placed at 4°C for 1 2h and then incubated at 35°C for 18 24h. The discs were tested in triplicate. At the end of the period, the inhibition zones formed on the media were measured with a transparent ruler in millimeters.

Determination of the minimum inhibitory concentration (MIC)

Five concentrations of each extract (3.125, 6.25, 12.5, 25 and 50 mg/ml) were prepared. The antimicrobial effect of each concentration was measured. The various concentrations were loaded onto 6 mm disks which were then pressed onto already prepared Mueller-Hinton agar plates. The inoculated plates were incubated at 35°C for 48h. MICs were determined after 24h for the bacteria. Zones of inhibition were measured at the end of the incubation period. The MICs were determined as the lowest concentrations of extracts inhibiting the visible growth of each organism on the agar plate.

RESULTS

The results of the antimicrobial effects of the different plant extracts are shown in table 1. The different plant parts were categorized as dichloromethane, methanol, ethanol, hexane and aqueous and coded as shown in column 1. For full detail of the code, see the abbreviations. The dichloromethane extracts showed some form antibacterial activity albeit a weak one. The dichloromethane root extracts of *C. volkensii* was the most active of all the dichloromethane extract showing a broad spectrum antibacterial activity against the tested microbes than the rest of dichloromethane extracts. Dichloromethane root extracts of *A. setaceous* exhibited greater antibacterial activity against *B. subtilis* and *S. faecalis* but had very weak antibacterial activity against *E. coli* and *P. aeruginosa* while it lacked activity against *S. aureus*. The most susceptible microorganism to dichloromethane extracts was the *B. subtilis* whereas *S. aureus* and *P. aeruginosa* seems to be resistant to most of the extracts.

The methanolic extracts also exhibited some form of antimicrobial activity against the tested microbes. The methanolic leaves extract of *C. volkensii* was active against *B. subtilis*, *P. aeruginosa*, *S. faecalis*, and *S. aureus* but completely lacked activity against *E. coli*. The methanolic extract of *C. volkensii* stem was more active against *B. subtilis* but had weaker activity against the rest of the microbes. The same results were observed with the methanolic extracts of *C. volkensii* root. The methanolic extract of *A. setaceous* aerial part had broad antibacterial activity against all the microbes tested. Methanolic extract of *A. setaceous* root was more active against *B. subtilis* and *S. faecalis* but lacked activity against *E. coli*. *B. subtilis* was the most susceptible microorganism while *E. coli* and *S. aureus* were the least susceptible. *C. volkensii* leaf seemed to be the most active plant part.

The ethanolic extract of *C. volkensii* and *A. setaceous* exhibited various degree of antibacterial. The ethanol extract of *C. volkensii* leaf showed weak activity against *E. coli*, *S. aureus*, *B. subtilis*, *S. faecalis*. *P. aeruginosa* was resistant to the ethanolic

leaf extract of *C. volkensii*. The ethanolic stem extract of *C. volkensii* also showed weak activity against the tested microbes but was inactive against *P. aeruginosa*. The ethanolic root extracts of *C. volkensii* demonstrated weak activity against all the tested bacteria whereas the ethanolic extract of *A. setaceous* aerial part was particularly active against *E. coli* but showed weak activity against *S. aureus*, *B. subtilis* and *S. faecalis*. It was inactive against *P. aeruginosa*. The ethanolic root extract of *A. setaceous* was found to show some form of activity against *E. coli*, *S. aureus*, *B. subtilis*, *S. faecalis*. It was inactive against *P. aeruginosa*. The least susceptible microorganism to methanolic extracts of the two plants was the *P. aeruginosa* while the most susceptible was *B. subtilis*. Ethanolic root extract of *C. volkensii* exhibited broad spectrum activity showing some form of activity against all the tested microbes.

The hexane extracts of *C. volkensii* and *A. setaceous* exhibited antibacterial activity against all the tested microbes. Leaf, stem and root of *C. volkensii* showed activity against the microbes with the highest activity being demonstrated by the root extracts of *C. volkensii* against *B. subtilis* and *S. faecalis*. The hexane extracts of aerial part and root of *A. setaceous* were generally active against all the tested microbes with the highest activity being shown by the root extract of *A. setaceous* against *B. subtilis* and *S. faecalis*. The most susceptible microorganism to hexane extracts were *B. subtilis* and *S. faecalis* whereas the most resistant microbe to the hexane extracts was *E. coli*. The hexane leaf extract of *C. volkensii* seemed to be the most active plant part showing activity against most of the bacteria.

The minimum inhibitory concentrations of *C. volkensii* and *A. setaceous* extracts

The minimum inhibitory concentration (MIC) values of the plant extracts were also investigated. MIC was taken to be the lowest concentration of the extracts that completely inhibited the growth of the microorganism. Data are reported as mean inhibition zones (mm) ± SEM of three replicates. Dichloromethane leaf extracts of *C. volkensii* had a MIC value ranging from 12.5mg/ml to 25mg/ml. With *E. coli*, *S. aureus*, *B. subtilis* it had a MIC value of 12.5mg/ml while with *P. aeruginosa* and *S. faecalis* had MIC values of 25mg/ml. The dichloromethane extracts of *C. volkensii* stem showed MIC values ranging from 12.5mg/ml for *S. aureus* and *B. subtilis* to 25mg/ml for *E. coli*, and *S. faecalis.* The extracts were largely inactive against *P. aeruginosa* at all the concentrations tested. The dichloromethane extracts of *C. volkensii* root were active at most of the concentrations tested with MIC ranging from 3.2mg/ml against *E. coli*, 6.25mg/ml for *S. aureus* to 12.5mg/ml against *B. subtilis*, *P. aeruginosa* to 25mg/ml for *S. faecalis*. Dichloromethane extract of aerial part of *A. setaceous* had high MIC values ranging from 25mg/ml to 50mg/ml with MIC value of 25mg/ml for *E. coli*, *B. subtilis*, and *S. faecalis*. The extracts lacked activity against *S. aureus* even at the highest concentration whereas it was only active against *P. aeruginosa* at the highest concentration. Dichloromethane root extract of *A. setaceous* had MIC values of between 6.25mg/ml and 50mg/ml. The MIC values were 6.25mg/ml for *E. coli* and *B. subtilis*, 12.5mg/ml for *S. faecalis*, 25mg/ml for *P. aeruginosa*. The MIC for *S. aureus* was 50mg/ml (Table 2a).

For the methanolic extracts, the *C. volkensii* leaf had MIC ranging from 12.5 to 50mg/ml with MIC of 12.5mg/ml for *B. subtilis* and 25mg/ml for *S. aureus*, *P. aeruginosa*, and *S. faecalis*. The extract was only active against *E. coli* at the highest concentration tested (50mg/ml). The MIC values ranged from 6.25 to 25mg/ml for the methanolic extract of *C. volkensii* stem. The extract had MIC value of 6.25mg/ml for *S. aureus* and *B. subtilis* and 25mg/ml for *E. coli*, *P. aeruginosa*, and *S. faecalis*. *C. volkensii* root extracts MIC values ranged from 6.25 to 25mg/ml with low MIC for *B. subtilis* at 6.25mg/ml, and 12.5 and 25mg/ml for *S. aureus*, *P. aeruginosa* and *E. coli*, and *S. faecalis* respectively. Methanolic extract of *A. setaceous* aerial part had MIC values of 6.25mg/ml for *B. subtilis*, 12.5mg/ml for *E. coli*, *S. aureus*, and *P. aeruginosa*. For *S. faecalis*, it was 25mg/ml. For *A. setaceous* root extract, *B. subtilis* had a low MIC value of 6.25mg/ml followed by 12.5mg/ml for *S. aureus* and 25mg/ml for *P. aeruginosa*, and *S. faecalis*. The root ethanolic extract lacked activity against *E. coli* even at the highest concentration tested (Table 2b).

The MIC values for the ethanolic extracts of *C. volkensii* leaf were 6.25mg/ml for *S. aureus*, 12.5mg/ml for *B. subtilis* and 25mg/ml for *E. coli*, *P. aeruginosa*, and *S. faecalis*. For the ethanolic stem extracts of *C. volkensii*, the values were 6.25mg/ml for *B. subtilis* and *S. faecalis* and 25mg/ml for *E. coli*, and *P. aeruginosa*. With the root extracts of *C. volkensii*, MIC values were 6.25mg/ml for *S. aureus*, 12.5mg/ml for *E. coli* and *B. subtilis* and 25mg/ml for *P. aeruginosa*, and *S. faecalis*.

The MIC values for ethanolic extracts of *A. setaceous* aerial part ranged from 3.2mg/ml for *S. aureus*, 6.25mg/ml for *E. coli* and 25mg/ml for *B. subtilis*, *P. aeruginosa*, and *S. faecalis* while for the ethanolic root extracts of the same plant, MIC ranged from 6.25mg/ml for *S. aureus* and *B. subtilis* to 25mg/ml for *E. coli*, *P. aeruginosa*, and *S. faecalis* (Table 2c)

The hexane extracts of both *C. volkensii* and *A. setaceous* had low MIC with values ranging from 3.2mg/ml to 25mg/ml. For the hexane leaf *C. volkensii* extract, it was 3.2mg/ml for *E. coli*, 6.25mg/ml for *S. aureus*, 12.5mg/ml for *B. subtilis* and 25mg/ml for *P. aeruginosa* and *S. faecalis*. With the stem extracts of *C. volkensii*, MIC ranged from 12.5mg/ml for *S. aureus* and *B. subtilis* to 25mg/ml for *E. coli*, *P. aeruginosa* and *S. faecalis*. For the hexane root extract of *C. volkensii*, MIC ranged from 12.5mg/ml for *S. aureus*, *B. subtilis* and 25mg/ml for *E. coli*, *P. aeruginosa* and *S. faecalis*. The MIC values for hexane *A. setaceous* aerial part were 12.5mg/ml for *S. aureus* and *B. subtilis* and 25mg/ml for *E. coli*, *P. aeruginosa*, and *S. faecalis*, while the same values for hexane root extracts of *A. setaceous* were 6.25mg/ml for *S. aureus*, *B. subtilis*, and 12.5mg/ml for *S. faecalis* and 25mg/ml for *E. coli*, and *P. aeruginosa* (Table 2d).

From the above result, it is apparent that gram positive organisms were generally more susceptible to the various plant extracts at lower concentration when compared to gram negative bacteria.

DISCUSSION

Traditional herbal cures and remedies have played an important historical role in the treatment of a variety of illness and diseases for the last three hundred years [13]. Nature has been a source of medicinal agents for thousand of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Higher plants, as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Plants with possible antimicrobial

Table 1: Antimicrobial effects of the various parts of the plant extracted with different solvents. All values are expressed as mean inhibition zones (mm) ± SEM of three replicates

Group	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*
DCVL	2.67±0.33	1.67±0.33	4±0.58	1.67±0.33	2±0.58
DCVS	2±0.58	3.33±0.88	3.33±0.33	-	4±0.58
DCVR	3.33±0.67	4±0.58	5±0.58	2.33±0.33	2±0.00
DASA	0.67±0.33	-	2±0.58	-	1.67±0.33
DASR	2±0.58	-	4.33±0.33	-	5.67±0.33
MCVL	-	2±0.58	4±0.58	4±0.58	4±0.58
MCVS	2.33±0.33	1.33±0.33	4±1.00	1.33±0.33	4.33±0.33
MCVR	2±0.58	1.33±0.33	5±0.58	1.33±0.33	3.33±0.67
MASA	2.7±0.58	1.3±0.58	2.7±0.58	3.3±0.58	2.7±0.33
MASR	-	2±1.15	5.33±0.33	1.67±0.67	4±0.58
ECVL	2±0.58	1±0.00	2.67±0.67	-	3.33±0.33
ECVS	1.67±0.33	1.33±0.33	5.33±0.67	-	5.33±0.33
ECVR	2.67±0.33	0.67±0.33	4.33±0.67	1.33±0.33	5±0.58
EASA	6.33±0.33	1.67±0.33	1.33±0.33	-	3±0.00
EASR	1.67±0.33	2.33±0.33	8.33±0.33	-	3.67±0.33
HCVL	4±0.58	1±0.00	4±0.58	4±0.58	4±0.00
HCVS	0.33±0.33	1.33±0.33	5.33±0.33	1.67±0.33	4.33±0.33
HCVR	1.67±0.33	1.67±0.33	6±0.58	2.33±0.33	5.33±0.33
HASA	1.33±0.33	1.6±0.33	3.33±0.33	2.33±0.33	3.6±0.67
HASR	2.33±0.33	1.33±0.33	6.67±0.33	1.33±0.33	5.67±0.33

Table 2(a): Minimum Inhibitory concentration of dichloromethane extracts of *C. volkensii* and *A. setaceous*.

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*
DCVL	50	4.67±0.33	2.67±0.33	4.33±0.33	2.33±0.33	1.67±0.33
	25	3±0.58	3±0.00	2±0.58	1.67±0.33	1.33±0.33
	12.5	1.33±0.33	1.33±0.33	1.67±0.33	-	-
	6.25	-	-	-	-	-
	3.2	-	-	-	-	-
DCVS	50	4±0.58	3.33±0.33	4.33±0.33	-	4±0.58
	25	2±0.58	2.33±0.33	3.33±0.33	-	2±0.58
	12.5	-	1.33±0.33	1.33±0.33	-	-
	6.25	-	-	-	-	-
	3.2	-	-	-	-	-
DCVR	50	5.67±0.33	4.67±0.33	5.33±0.67	5.33±0.33	3.33±0.33
	25	3.67±0.33	3.67±67	5±0.58	3.33±0.33	1.67±0.33
	12.5	2.67	2.33±0.33	2±0.58	2.33±0.33	-
	6.25	1.33±0.33	1.33±0.33	-	-	-
	3.2	1±0.00	-	-	-	-
DASA	50	1.33±0.33	-	4±0.58	2±0.58	2.33±0.33
	25	1±0.00	-	2±0.58	-	1.33±0.00
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
	3.2	-	-	-	-	-
DASR	50	2.67±0.33	1.33±0.58	7.33±0.33	3.33±0.33	5.67±0.33
	25	2±0.58	-	5±0.58	2.33±0.33	5±0.58
	12.5	1±0.00	-	2±0.58	-	2.33±0.33
	6.25	1±0.00	-	1.33±0.33	-	-
	3.2	-	-	-	-	-

Table 2(b): Minimum Inhibitory concentration of methanolic extracts of *C. volkensii* and *A. setaceous*.

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*
MCVL	50	2±0.58	2.33±0.33	4±0.58	3.67±67	4±0.58
	25	-	1.33±0.33	2.67±0.33	2±0.58	1.67±0.33
	12.5	-	-	1.33±0.33	-	-
	6.25	-	-	-	-	-
	3.2	-	-	-	-	-
MCVS	50	5.33±0.33	3±0.58	4±1	3.33±0.33	4.33±0.33
	25	4.33±0.33	1.67±0.33	3±0.58	1.67±0.33	3.33±0.33
	12.5	-	1.33±0.33	1.67±0.33	-	-
	6.25	-	1.00±0.00	1.33±0.33	-	-
	3.2	-	-	-	-	-
MCVR	50	3.33±0.33	1.67±0.33	6±0.58	4.67±0.33	3.67±0.33
	25	2±0.58	1.33±0.33	4.33±0.33	3.67±0.33	3±0.58
	12.5	-	1±0.00	2.33± 0.33	2±0.58	1.33±0.33
	6.25	-	-	1.67±0.33	-	-
	3.2	-	-	-	-	-
MASA	50	5.33±0.33	3.33±0.33	3±0.58	4.33±0.33	3.33±0.33
	25	3.67±0.33	1.33±0.33	2.67±0.33	3.67±67	2.67±0.33
	12.5	2.33±0.33	1±0.00	1.67±0.33	1.67±0.33	-
	6.25	-	-	1±0.00	-	-
	3.2	-	-	-	-	-
MASR	50	-	3.67±0.33	5±0.58	4±0.58	3.67±0.33
	25	-	2.67±0.33	3±0.58	1.67±0.33	3±0.58
	12.5	-	1.33±0.33	2.00±0.58	-	-
	6.25	-	-	1.33± 0.33	-	-
	3.2	-	-	-	-	-

Table 2(c): Minimum Inhibitory concentration of ethanolic extracts of *C. volkensii* and *A. setaceous*.

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*
ECVL	50	2.67±0.33	4±0.58	4.33±0.67	3.33±0.33	3.33±0.33
	25	2±0.58	2.33±0.33	2.33±0.33	3±0.58	2.67±0.33
	12.5	-	1.33±0.00	1.33±0.33	-	-
	6.25	-	1±0.00	-	-	-
	3.2	-	-	-	-	-
ECVS	50	2.33±0.33	4.33±0.33	4.67±0.67	1.33±0.33	5.33±0.33
	25	1.67±0.33	2±0.58	2.67±0.67	1.33±0.33	4±0.58
	12.5	-	2±0.33	1.33±0.33	-	1.67±0.33
	6.25	-	1±0.33	-	-	-
	3.2	-	-	-	-	-
ECVR	50	4.33±0.33	4.33±0.33	4.33±0.58	2±0.58	5.67±0.33
	25	2.67±0.33	2.33±0.33	4.33±0.33	1.33±0.33	3±0.58
	12.5	2±0.00	1.67±0.33	2.00±0.33	-	-
	6.25	-	1±0.00	-	-	-
	3.2	-	-	-	-	-
EASA	50	7.33±0.33	2.33±0.33	2.67±0.33	1.33±0.33	2.67±0.33
	25	6.67±0.33	2±0.00	1.33±0.00	1.33±0.33	1.67±0.33
	12.5	4.67±0.33	4.33±0.33	-	-	-
	6.25	1.67±0.33	3.00±0.33	-	-	-
	3.2	-	2.67±0.33	-	-	-
EASR	50	4.67±0.33	5.33±0.33	8.33±0.33	2.67±0.33	3.67±0.33
	25	2.67±0.33	3.33±0.33	6.33±0.67	2.33±0.33	2.00±0.33
	12.5	-	1.67±0.33	3±0.58	-	-
	6.25	-	1±0.00	1.67 0.33	-	-
	3.2	-	-	-	-	-

Table 2(b): Minimum Inhibitory concentration of methanolic extracts of *C. volkensii* and *A. setaceous*.

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*
HCVL	50	6.33±0.33	3.33±0.33	4.33±0.33	4±0.58	4±0.00
	25	5.67±0.33	2.67±0.33	3.33±0.33	3.33±0.33	1.33±0.33
	12.5	3.67±0.33	1.67±0.33	1.67 ±0.33	-	-
	6.25	3.33±0.33	1.33±0.33	-	-	-
	3.2	1.67±0.33	-	-	-	-
HCVS	50	2.67±0.33	3.67±0.33	5±0.58	2.67±0.33	4±0.58
	25	1.33±0.33	2.67±0.33	4.67±0.67	2.33±0.33	2.67±0.33
	12.5	-	1.67±0.33	1.33±0.33	-	-
	6.25	-	-	-	-	-
	3.2	-	-	-	-	-
HCVR	50	2.33±0.33	3.33±0.33	5.33±0.88	2.67±0.33	4.33±0.33
	25	2.±0.58	3.33±0.33	3.67±0.33	1.67±0.33	3.67±0.33
	12.5	-	1.67±0.33	1.33±0.33	-	-
	6.25	-	-	-	-	-
	3.2	-	-	-	-	-
HASA	50	2.33±0.33	3.33±0.33	2.67±0.33	2.33±0.33	4.33±0.33
	25	1.33±0.33	1.67±0.33	1.67±0.33	1.67±0.33	1.67±0.33
	12.5	-	1.33±0.33	1.33±0.33	-	-
	6.25	-	-	-	-	-
	3.2	-	-	-	-	-
HASR	50	4.67±0.33	3±0.58	7.33±0.33	4±0.58	5.33±0.67
	25	2.67±0.33	1.67±0.33	5.67±0.88	1.67±0.33	4.33±0.33
	12.5	-	1.33±0.33	2±0.58	-	2.33±0.33
	6.25	-	1±0.00	1.33±0.33	-	-
	3.2	-	-	-	-	-

activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The selection of crude plant extracts for screening program as the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products [1]. The antibacterial activities of *A. setaceous* and *C. volkensii* are reported for the first time. No previous report on the antibacterial activity of these species could be found in the literature.

In the present study, dichloromethane extracts showed some form of antibacterial activity. The dichloromethane root extracts of *C. volkensii* was the most active of all the dichloromethane extract showing a broad spectrum antibacterial activity against the tested microbes than the rest of dichloromethane extracts. Dichloromethane root extracts of *A. setaceous* exhibited greater antibacterial activity against *B. subtilis* and *S. faecalis* but had very weak antibacterial activity against *E. coli* and *P. aeruginosa* while it lacked activity against *S. aureus*. The most susceptible microorganism to dichloromethane extracts was the *B. subtilis* whereas *S. aureus* and *P. aeruginosa* seems to be resistant to most of the extracts. It would appear that the dichloromethane roots extracts of both *C. volkensii* and *A. setaceous* were the most active of the dichloromethane with the *C. volkensii* root showing broad spectrum activity against both the gram positive and negative bacteria. Alkaloids, flavonoids and tannins have been reported to possess antibacterial activity and it would therefore suffice to say that the antibacterial activity shown by some of these extracts could be attributed to these compounds. It is difficult to explain why some of the extracts were active against some strain of the gram positive bacteria and not others as well as being active against some gram negative and not all. The observed resistance of some of the gram positive and gram negative probably could be due to cell permeability or due to other genetic factors.

The methanolic extracts also exhibited some form of antimicrobial activity against the tested microbes. The methanolic leaf extract of *C. volkensii* was active against *B. subtilis*, *P. aeruginosa*, *S. faecalis*, and *S. aureus* but completely lacked activity against *E. coli*. The methanolic extract of *C. volkensii* stem was more active against *B. subtilis* but had weaker activity against the rest of the microbes. The same results were observed with the methanolic extracts of *C. volkensii* root. The methanolic extract of *A. setaceous* aerial part had broad antibacterial activity against all the microbes tested. Methanolic extract of *A. setaceous* root was more active against *B. subtilis* and *S. faecalis* but lacked activity against *E. coli*. *B. subtilis* was the most susceptible microorganism while *E. coli* and *S. aureus* were the least susceptible. *C. volkensii* leaf seemed to be the most active plant part. It is therefore possible to postulate that antibacterial activity seen with the methanolic extracts may have been due to the presence of alkaloid, flavonoids and tannins. Flavonoids' activity is probably due to their ability to form complex with extra cellular and soluble proteins, as well as bacterial cell wall. Lipophilic flavonoids may also disrupt bacterial membranes. *T. pubescens* methanolic extract was demonstrated to possess antibacterial activity against Gram positive bacteria including *S. aureus*, MRSA, *Streptococcus pyogenes* and *E. faecalis* but not against Gram negative *E. coli*, *P aeruginosa* and S*almonella* spp [14]. Gram negative organisms tend to have a higher intrinsic resistance to most antimicrobial agents (15). The roots and root bark methanolic extracts of *A.*

africanus and *A. racemosa* were demonstrated to be active against *Staphylococcus aureus* and *Neisseria gonorrhea* respectively but lacked activity against S*higella boydii* and *P. aeruginosa*. The same study also showed that *Tamarindus indica* (Caesalpinaceae) leaves methanolic extracts were active against *Shigella boydii* and *P. aeruginosa* but not against *S. aureus* and *N. gonorrhea* [5]. The methanolic leaf extracts of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cardifolia*, *Withania somnifer* and *Ziziphus mauritania* showed significant antibacterial activity against *B. subtilis*, *E. coli*, *Pseudomonas fluorescens*, S*taphylococcus aureus* [16]. *Sida cordifolia* showed significant antibacterial activity against *B. subtilis* and *S. typhi* while *Mimosa pudica* and *Aegle marmelos* were found to be active against *B. subtilis*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. coli* and *S. typhi* with maximum activity noted against *P. aeruginosa* and *S. typhi* [17].

The ethanolic extract of *C. volkensii* and *A. setaceous* exhibited various degree of antibacterial. The ethanol extract of *C. volkensii* leaves showed weak activity against *E. coli*, *S. aureus*, *B. subtilis*, *S. faecalis* and *C. albicans*. *P. aeruginosa* was resistant to the ethanolic leaves extract of *C. volkensii*. The ethanolic stem extract of *C. volkensii* also showed weak activity against the tested microbes but was inactive against *P. aeruginosa*. The ethanolic root extracts of *C. volkensii* demonstrated weak activity against all the tested bacteria and fungus whereas the ethanolic extract of *A. setaceous* aerial part was particularly active against *E. coli* but showed weak activity against *S. aureus*, *B. subtilis* and *S. faecalis*. It was inactive against *P. aeruginosa*. The ethanolic root extract of *A. setaceous* was found to show some form of activity against *E. coli*, *S. aureus*, *B. subtilis*, and *S. faecalis*. It was inactive against *P. aeruginosa*. The least susceptible microorganism to methanolic extracts of the two plants was the *P. aeruginosa* while the most susceptible was *B. subtilis*. Ethanolic root extract of *C. volkensii* exhibited broad spectrum activity showing some form of activity against all the tested microbes.

The hexane extracts of *C. volkensii* and *A. setaceous* exhibited antibacterial and antifungal activity against all the tested microbes. Leaf, stem and root of *C. volkensii* showed activity against the microbes with the highest activity being demonstrated by the root extracts of *C. volkensii* against *B. subtilis* and *S. faecalis*. The hexane extracts of aerial part and root of *A. setaceous* were generally active against all the tested microbes with the highest activity being shown by the root extract of *A. setaceous* against *B. subtilis* and *S. faecalis*. The most susceptible microorganism to hexane extracts were *B. subtilis* and *S. faecalis* whereas the most resistant microbe to the hexane extracts was *E. coli*. The hexane leaf extract of *C. volkensii* seemed to be the most active plant part showing activity against most of the bacteria.

The secondary metabolites present in the plant materials used in this study could be responsible for antimicrobial activity exhibited by some of the extracts of these two plants. There antibacterial activity may be indicative of the presence of some metabolic toxins or broad-spectrum antibiotics. Several metabolites from herb-species, such as alkaloids, tannins, saponins and sterols have been previously associated with antimicrobial activity [18]. Many studies indicate that in some plants, there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, phenols and water, ethanol, chloroform and butanol soluble compounds. Many plants contain non toxic glycosides which can get hydrolysed to release phenolics which are toxic to microbial pathogens [2]. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. Gram positive bacteria were found to be more susceptible than gram negative bacteria. This could be due to the fact that the cell wall of gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope whereas Gram negative bacteria cell wall is a multi layered structure bounded by an outer cell membrane [19]. The most sensitive bacterium was *B. subtilis* which was inhibited by all the extracts. It is also not surprising that there are differences in the antibacterial effects of plant extracts, which could have been due to the phytochemical differences between the plant extracts. It therefore suggests that constituents of the plant extracts could serve as source of drugs useful in the chemotherapy of some microbial infections.

The active components usually interfere with growth and metabolism of microorganisms in a negative manner and are quantified by determining the minimum inhibitory concentration. These suggest that they were bacteriostatic. Most of the extracts had a minimum inhibitory concentration ranging from 3.2mg/ml to 50mg/ml which indicated that the extracts had the ability to interfere with the growth of some of the bacteria considerably slowing their growth rates.

This work showed that some of the extracts can be used as broad spectrum antibiotics since they are both active against both Gram positive and negative bacteria. Antibacterial effects of these plants on *S. aureus*, *E. coli* and *P. aeruginosa* showed that the plant extracts can be used in the treatment of gastrointestinal infection, urinary tract infection and diarrhea in man and skin diseases. They could also be used in treatment of boils, sores and wounds associated with *S. aureus* and *P. aeruginosa* that have been implicated as causative agents of these diseases [2,] [20].

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