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Growth Inhibitory Effects of Solvent Extracts of Selected Plants on ß-Lactamase Producing Bacteria

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Abstract: Components of the stem-barks and stem of four different plants, reputed to be medicinal in Northern Nigeria in the treatment of genitourinary tract infections were extracted using 95% ethanol. Ethanol extracts obtained from parts of plants were partitioned using chloroform, distilled water, ethyl acetate, methanol and petroleum ether solvents of varying polarity indices in to fractions which were later screened together with saved ethanol extracts against ß-lactamase producing bacteria that have demonstrated some resistance to ß-lactam antibiotics. The screened extracts and fractions of both the stem-barks and stem of *Butyrospermum parkii*, *Kigelia pinnata* and *Maytenus senegalensis* inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia* which are causative agents of genitourinary tract infections in the paper disk-plate method employed in the investigation. This finding is in support of the ethno-medicinal uses of these plants. On a contrary, the ethanol extract of the stem of Anogeissus leiocarpus showed no growth inhibition on the five bacterial isolates.

Key words: *Anogeissus leiocarpus*, *Butyrospermum parkii*, *Kigelia pinnata*, *Maytenus senegalensis*, toxic component, growth inhibition, clinical isolates, folkloric or ethno-medicinal uses

INTRODUCTION

Plants have served as sources of drugs and pharmaceuticals for man and other animals from time immemorial. There are about half a million plants now growing on earth, many of which possess therapeutic and pharmaceutical properties (Sanberg and Bruhn, 1979). According to an earlier survey, about 25% of modern drugs and medicinal products are derived from plant secondary metabolites (Muller, 1973). Penicillin G or benzylpenicillin, a drug isolated from the mould Penicillium notatum has been active against ß-lactamase producing bacteria and it is recently being resisted by the enzyme ß-lactamase secreted by these bacteria. This has therefore posed the need for the search of antimicrobials with different activity profiles (Karaman *et al*., 2003). Currently, the biological activity and importance of secondary metabolites of some plants which support their folkloric uses are not fully or scientifically established, thus requiring more studies to be carried out. As a further development on this, *Anogeissus leiocarpus*, *Butyrospermum parkii*, *Kigelia pinnata* and *Maytenus senegalensis* were selected for investigation of their bioactive components which could be used to combat diseases (Richard, 1998; Ghazi *et al*., 1999).

The five clinical isolates used in this study are known to be disease causing agents and as earlier mentioned have proved resistance to benzylpenicillin. For instance, *Staphylococcus aureus* is often connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis and furuncle etc., Pseudomonas aeruginosa and Proteus vulgaris for cystitis, pyelitis and urethritis, *Escherichia coli* for enteritis and diarrhea and Klebsiella species for Pneumonia (Komolafe and Adegoke, 2008).

MATERIALS AND METHODS

Plant materials: The stem-barks and stems of plants from four families were chosen on the basis of folkloric uses that suggest their toxicity to bacteria. Specimens were collected at random from Kaduna (Zaria), Bauchi and Kano States of Nigeria, identified by Baba Ali and authenticated by B.S. Aliyu both of the Biological Science Department, Bayero University Kano, Nigeria. The places and dates of collection of plant parts together with their voucher specimen numbers are shown in Table 1.

Extraction procedure: Air dried and ground parts of plant materials were extracted by percolation with 95% EtOH at room temperature for two weeks. 500 ml of EtOH was used in each instance to percolate *B. parkii* and *M. senegalensis*. 700 ml of the same solvent was used for the percolation of *K. pinnata*, whereas for *A. leiocarpus* 1000 ml of the solvent was used. In the end of the two weeks interval, percolates were evaporated to dryness on a rotary evaporator (R 110) at 40°C and EtOH extracts obtained were weighed and labeled F_{001}.

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Table 1: Families, names (scientific and local), parts, places and dates of collection and herbarium voucher numbers of plants under investigation

Plant name and family	Local name(s) in Hausa	Part(s) used	Place and date of collection	Voucher No.
Anogeissus leiocarpus DC (Combretaceae)	Marke	Stem-bark	Gwarzo town, Kano State. August, 2002	4/70
Butyrospermum parkii Kotschy (Sapotaceae)	Kadanya	Stem-bark	Zaria,Kaduna State. August, 2002	5/60
Kigelia pinnata Syn (Bignoniaceae)	Nonongiwa Raehana	Stem-bark	Yankari village 70 km South of Bauchi town, Bauchi State. August, 2002	80/193
Maytenus senegalensis Lam (Celastraceae)	Namijin Tsada	Stem	Zaria, Kaduna State. August, 2002	40/246

Portions of EtOH extracts prepared as mentioned above were transferred in to vials and kept for antibacterial tests while the remainders were partitioned between $CHCl_3$ and distilled H_2O solvents (200ml,1:1), with the aid of separating funnels. $CHCl_3$ soluble fractions, F_{002} and interface fractions between $CHCl_3$ and distilled H_2O solvents, F_{001*} (where applicable), were separately evaporated to dryness on a rotary evaporator at 40°C, whereas distilled H_2O soluble fractions, F_{003} were washed several times with EtOAc (100 ml), which yielded distilled H_2O soluble fractions, F_{003}, interface fractions between distilled H_2O and EtOAc solvents, F_{002*} (where applicable) and EtOAc soluble fractions, F_{004}. These soluble solvent fractions were separated and concentrated as the $CHCl_3$ soluble fractions and the interface fractions between $CHCl_3$ and distilled H_2O solvents earlier discussed. Portions of $CHCl_3$ residues obtained as mentioned earlier were transferred in to vials and kept for antibacterial tests while the remainders, were further partitioned between MeOH and petroleum ether solvents (200,1:1), to solvent fractions which were separated and concentrated as the $CHCl_3$ soluble fractions and the interface fractions between $CHCl_3$ and distilled H_2O solvents earlier discussed to give the MeOH (F_{005}), the interface (F_{003*}) and the petroleum ether (F_{006}) residues respectively. All residues obtained in the process were transferred in to distinct vials and kept at the lower compartment of a refrigerator until they were required for use.

Antimicrobial bioassay: Five bacterial strains, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia* which are the causative agents of most genitourinary tract infections were tested for growth sensitivity using the paper disk-plate method on ingredients from plants reputed to be of medicinal value in Northern Nigeria. All the organisms, ß-lactamase producing bacteria were clinical isolates obtained from the Department of Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria. A sterile inoculating loop was used to transfer a portion of the colony of each isolate on to trypton soya broth (T.S.B) that was incubated at room temperature for three days. 0.1 ml of the broth was diluted with 1 ml of distilled water in a ratio of 1:100 (Adoum *et al*., 1997).

A paper punch was used to prepare disks of about 6 mm diameter from Whatman number 1 filter paper. Batches of prepared disks were transferred in to a screw-cap bottle and sterilized in an oven at 140°C for 60 min.

The stock solution for the bioassay was prepared by dissolving 10 mg of each extract and fraction in 1 ml of Dimethylsulphoxide (DMSO) i.e. 10000 µg/ml. Concentrations of 5,000, 2,000 and 1,000 µg/ml of the plant extracts and fractions were prepared using DMSO and the stock solution already formed. 0.1 ml of each concentration of extracts and fractions was introduced in to labeled screw-cap bottles containing 10 disks. The prepared disks were then kept in a refrigerator until required for use.

Disk-diffusion method or paper disk-plate method was employed. Sabouroud's Dextrose Agar (S.D.A) plates were inoculated with standard test inocula by direct streaking. The prepared disks were then introduced on to the inoculated surfaces and the plates were incubated at room temperature for 48 h (Pelczar *et al*., 1993).

Cultures were examined for areas of no growth around the disks (zones of inhibition). Organisms sensitive to the test extracts were inhibited at a distance from the disks, whereas resistant strains grew up to the edge of the disks. In this method, disks impregnated only with D.M.S.O served as control disks (Cheesbrough, 2000). Diameters of zones of inhibition were measured in millimeters with a ruler and recorded as sample means as shown in Table 2, 3a, 3b, 4a, 4b, 5a and 5b.

RESULTS AND DISCUSSION

Results in Table 2 illustrate the ineffectiveness of the EtOH extract of the stem-bark of *Anogeissus leiocarpus* in inhibiting the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*. This extract showed no zones of inhibition at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml on the five clinical isolates and it is an indication that the components of the stem of *Anogeissus leiocarpus* are non-toxic to the bacteria employed in the investigation. Hence, the investigation does not support the folkloric or ethno-medicinal uses of the plant.

Of all the results presented in Table 3a, the $CHCl_3$, the $CHCl_3$-distilled H_2O interface and the EtOAc fractions of the stem-bark of Butyrospermum parkii recorded no inhibition of growth on *Staphylococcus aureus* at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. This same trend of no growth inhibition was demonstrated by the EtOH extract, the $CHCl_3$ and the EtOAc fractions of the stem-bark of the plant at lower

Table 2: Zones of inhibition (mm) exhibited by EtOH extract of the stem-bark of *Anogeissus leiocarpus* on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	Concentration (µg/ml)				
Bacterial isolate	1,000	2,000	5,000	10,000	Negative control
S. aureus	0	0	0	0	0
P. aeruginosa	0	0	0	0	0
E. coli	0	0	0	0	0
P. vulgaris	0	0	0	0	0
K. pneumoniae	0	0	0	0	0

concentrations of 1,000, 2,000 and 5,000 µg/ml but on *Pseudomonas aeruginosa*. On a contrary, these soluble solvent extracts or fractions of the stem-bark of *Butyrospermum parkii* exhibited growth inhibitory effects on this clinical isolate at the concentration of 10,000 µg/ml. The EtOH extract of the stem-bark recorded a mean zone of inhibition of 7±0.17 on *Pseudomonas aeruginosa*, whereas the $CHCl_3$ and the EtOAc fractions recorded mean zones of inhibition of 7.03±0.3 and 8±0.1 respectively. At the respective concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml, the EtOH soluble extract demonstrated an increasing trend of growth inhibition on *Staphylococcus aureus* with mean zones of inhibition of 1.4±0.1, 2±0.2, 5.5±0.2 and 7±0.2. A similar trend of increasing growth inhibition was exhibited by the distilled H_2O fraction of the stem-bark of the plant on both *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical isolates. The mean zones of inhibition recorded in an increasing order as the tested concentrations were 1.6±0.3, 2±0.2, 4±0.1 and 7±0.1 for *Staphylococcus aureus* and 2.2±0.1, 3.1±0.12, 5.3±0.17 and 7±0.17 for *Pseudomonas aeruginosa*.

The overall results in Table 3a revealed that the EtOH extract, the $CHCl_3$, the distilled H_2O and the EtOAc fractions of the stem-bark of *Butyrospermum parkii* contained toxic components which inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This research finding conforms with the folkloric or ethno-medicinal uses of the plant.

A comprehensive study of the results recorded in Table 3b for the growth inhibitory effect exhibited by extracts of the stem-bark of *Butyrospermum parkii* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae* revealed that the distilled H_2O and EtOAc fractions of the stem-bark of the plant were non-toxic to the three clinical isolates at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The $CHCl_3$ fraction, at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml was non-toxic to *Proteus vulgaris* and *Klebsiella pneumonia* and recorded no zones of inhibition. A similar trend of no zones of inhibition was exhibited by the $CHCl_3$-distilled H_2O interface fraction on *E. coli* and *K. pneumoniae* at these same concentrations. At concentrations of 1,000, 2,000 and 5,000 µg/ml, the EtOH extract of the stem-bark of *Butyrospermum parkii* showed no growth inhibitory effect on *E. coli*, *P. vulgaris* and *K. pneumoniaer*, whereas at 10,000 µg/ml concentration there were recorded growth inhibitions. The respective mean zones of inhibition recorded for *E. coli*, *P. vulgaris* and *K. pneumoniae* were 7±0.17, 7±0.27 and 7±0.10. In a similar manner as the EtOH extract, the $CHCl_3$ fraction did not exhibit growth inhibition on *E. coli* at lower concentrations of 1,000, 2,000 and 5,000 µg/ml, while at a higher concentration of 10,000 µg/ml there was a growth inhibition of mean value of 7±0.17. Also, there was no growth inhibition exhibited by the $CHCl_3$-distilled H_2O interface fraction on *P. vulgaris* at 1,000 and 2,000 µg/ml concentrations. It was only at 5,000 and 10,000 µg/ml concentrations that this fraction demonstrated growth inhibition with mean zones of 7±0.27 and 8±0.10 respectively.

From the results recorded in Table 3b, it could be seen that the EtOH extract, the $CHCl_3$ and the $CHCl_3$-distilled H_2O interface fractions showed the presence of components in the stem-bark of the plant which inhibited the growth of *E. coli*, *P. vulgaris* and *K. pneumoniae*. This research finding is in support of the folkloric or ethno-medicinal uses of *Butyrospermum parkii*.

Results in Table 4a revealed that at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml, the $CHCl_3$, the distilled H_2O, the distilled H_2O-EtOAc interface, the EtOAc, the MeOH and the petroleum ether fractions of the stem-bark of *Kigelia pinnata* exhibited no growth inhibitory effect on *S. aureus* and *P. aeruginosa*. All the negative controls presented a similar trend of no growth inhibitory effect on the two clinical isolates. It is the EtOH extract of the plant which demonstrated growth inhibition on *S. aureus* at concentrations of 5,000 and 10,000 µg/ml with mean zones of inhibition of 7±0.10 and 8±0.3.0 respectively. At lower concentrations of 1,000 and 2,000 µg/ml, this solvent extract showed no growth inhibitory effect on the isolate. Also, there was no growth inhibition observed for *P. aeruginosa* at 1,000 µg/ml concentration of the solvent extract, but at higher concentrations of 2,000, 5,000 and 10,000 µg/ml the respective mean zones of inhibition shown were 2±0.10, 4.5±0.10 and 7±0.20.

The results in Table 4a clearly indicate that the stem-bark of *K. pinnata* contained components which inhibited the growth of *S. aureus* and *P. aeruginosa* when combined as shown by the EtOH extract and these components when separated or partitioned did not

Table 3a: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Butyrospermum parkii* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

	Staphylococcus aureus				*Pseudomonas aeruginosa*				
Solvent extract/	Concentration (µg/ml)				Concentration (µg/ml)				Negative
fraction	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Control
EtOH	1.4±0.10	2±0.20	3.5±0.20	7±0.20	0	0	0	7±0.17	0
$CHCl_3$	0	0	0	0	0	0	0	7.03±0.3	0
$CHCl_3$-H_2O	0	0	0	0	0	0	0	0	0
DistilledH_2O	1.6±0.30	2±0.20	4±0.10	7±0.10	2.2±0.1	3.1±0.12	5.3±0.17	7±0.17	0
EtOAc	0	0	0	0	0	0	0	8±0.10	0

Table 3b: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Butyrospermum parkii* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	P. vulgaris				*E. coli*				*K. pneumonia*				
	Concentration (µg/ml)				Concentration (µg/ml)				Concentration (µg/ml)				
Solvent extract/fraction	1000	2000	5000	10000	1000	2000	5000	10000	1000	2000	5000	10000	Negative Control
EtOH	0	0	0	7±0.27	0	0	0	7±0.17	0	0	0	7±0.10	0
$CHCl_3$	0	0	0	0	0	0	0	7±0.17	0	0	0	0	0
$CHCl_3$-DistilledH_2O	0	0	7±0.27	8±0.10	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4a: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Kigelia pinnata* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

	S. aureus				*P. aeruginosa*				
	Concentration (µg/ml)				Concentration (µg/ml)				
Solvent extract/fraction	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative Control
EtOH	0	0	7±0.10	8±0.30	0	2±0.10	4.5±0.10	7±0.20	0
$CHCl_3$	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0
H_2O-EtOAc	0	0	0	0	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0
MeOH	0	0	0	0	0	0	0	0	0
Petroleum Ether	0	0	0	0	0	0	0	0	0

Table 4b: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Kigelia pinnata* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	P. vulgaris				*E. coli*				*K. pneumoniae*				
Solvent extract/	Concentration (µg/ml)				Concentration (µg/ml)				Concentration (µg/ml)				
fractions	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative control
EtOH	2±0.30	3±0.10	5±0.20	7±0.10	1.5±0.10	2±0.10	3.4±0.10	7±0.20	0	2±0.10	5±0.20	8±0.30	0
$CHCl_3$	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H_2O-EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0
MeOH	0	0	0	0	0	0	0	0	0	0	0	0	0
Petroleum Ether	0	0	0	0	0	0	0	0	2.4±0.30	3±0.10	4.5±0.10	7±0.20	0

inhibit the growth of the two isolates as shown by the other solvent fractions of the plant.

Results shown in Table 4b clearly explain the growth inhibitory effect of extracts of the stem-bark of *Kigelia pinnata* on *E. coli*, *P. vulgaris* and *K. pneumoniae* at concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The EtOH extract, at these concentrations demonstrated growth inhibition with mean zones of inhibition of 2±0.30, 3±0.10, 5±0.20 and 7±0.10 on *P. vulgaris*. The trend of increasing growth inhibition as the concentration was also exhibited by this same extract on *E. coli*. The mean zones of inhibition exhibited were 1.5±0.10, 2±0.10, 3.4±0.10 and 7±0.20. For *K. vulgaris*, there was no inhibition of growth caused by the extract at 1,000 µg/ml concentration. A reverse of this occurred at higher concentrations of 2,000, 5,000 and 10,000 µg/ml with

Table 5a: Zones of inhibition (mm) exhibited by extracts of the stem of *Maytenus senegalensis* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

	S. aureus				*P. aeruginosa*				
	Concentration (µg/ml)				Concentration (µg/ml)				
Solvent extract/fractions	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative control
EtOH	0	0	0	0	0	0	0	7±0.17	0
$CHCl_3$	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0
Distilled H_2O-EtOAc	0	0	0	0	0	0	6±0.40	7±0.27	0
EtOAc	0	0	0	0	0	0	0	0	0

Table 5b: Zones of Inhibition (mm) exhibited by extracts of the stem of *Maytenus senegalensis* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	P. vulgaris				*E. coli*				*K. pneumonia*				
Solvent extract/	Concentration (µg/ml)				Concentration (µg/ml)				Concentration (µg/ml)				
fraction	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative control
EtOH	0	0	7±0.10	8±0.17	0	0	0	0	0	0	0	0	0
$CHCl_3$	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H_2O	2±0.20	3±0.20	7±0.10	8±0.10	0	7±0.10	8±0.10	0	0	0	0	0	0
Distilled H_2O-EtOAc	0	0	6±0.10	7±0.3	0	5.5±0.42	7±0.44	8±0.52	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0

mean zones of inhibition of 2±0.10, 5±0.20 and 8±0.30. Similarly, the $CHCl_3$, the distilled H_2O, the distilled H_2O-EtOAc interface, the EtOAc and the MeOH fractions of the stem-bark of the plant recorded no growth inhibition at all the tested concentrations on *E. coli*, *P. vulgaris* and *K. pneumoniae* just as they didn't on *S. aureus* and *P. aeruginosa* (Table 4a). Also, at the tested concentrations, the petroleum ether fraction demonstrated no growth inhibitory effect on *E. coli* and *P. vulgaris* just as it didn't on *S. aureus* and *P. aeruginosa* (Table 4a). It was only on *K. vulgaris* that this fraction exhibited growth inhibition with mean zones of inhibition of 2.4±0.30, 3±0.10, 4.5±0.10 and 7±0.20 at the respective concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml.

As drawn from the results given in Table 4b, it can be viewed that the EtOH extract and the petroleum ether fraction of the stem-bark of the plant contained components which are toxic that inhibited the growth of *E. coli*, *P. vulgaris* and *K. pneumoniae*. This finding serves as a back-up to the folkloric or ethno-medicinal uses of *Kigelia pinnata*.

For the results of mean zones of inhibition exhibited by extracts of the stem of Maytenus senegalensis on *S. aureus* and *P. aeruginosa* shown in Table 5a, it was only the EtOH extract and the distilled H_2O-EtOAc interface fraction that demonstrated growth inhibition on *P. aeruginosa*. The EtOH extract exhibited a mean zone of inhibition of 7±0.17 at 10,000 µg/ml concentration, while the distilled H_2O-EtOAc interface fraction presented mean zones of inhibition of 6±0.40 and 7±0.27 respectively at 5,000 and 10,000 µg/ml concentrations. For the $CHCl_3$, the distilled H_2O and the EtOAc fractions there were no growth inhibition recorded on both *S. aureus* and *P. aeruginosa* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. Also, the EtOH extract and the distilled H_2O-EtOAc interface fraction showed no growth inhibition on *S. aureus* at these concentrations. These extracts or fractions demonstrated no growth inhibition on *P. aeruginosa*. For the EtOH extract, there was no growth inhibition at 1,000, 2,000 and 5,000 µg/ml concentrations, whereas for the distilled H_2O-EtOAc interface fraction there was no growth inhibition only at 1,000 and 2,000 µg/ml concentrations.

From the results in Table 5a, it could be said that the EtOH extract and the distilled H_2O-EtOAc interface fraction of the stem of the plant contained components which are toxic that inhibited the growth of *P. aeruginosa*. This finding is in conformity with the folkloric or ethno-medicinal uses of *Maytenus senegalensis*.

A comprehensive analysis of the results in Table 5b revealed that the EtOH extract, the distilled H_2O and the distilled H_2O-EtOAc interface fractions of the stem of *Maytenus senegalensis* contained toxic components which inhibited the growth of *E. coli* and *P. vulgaris*, whereas the $CHCl_3$ and EtOAc fractions do not contain toxic components and did not inhibit the growth of the clinical isolates at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The EtOH extract inhibited the growth of *P. vulgaris* at 5,000 and 10,000 µg/ml concentrations with mean zones of inhibition of 7±0.10 and 8±0.17 respectively, whereas at lower concentrations of 1,000 and 2,000 µg/ml there were no growth inhibition. *E. coli* and *K. pneumoniae* experienced no growth inhibition from this extract at all the tested concentrations. The distilled H_2O fraction of the stem of *Maytenus senegalensis* demonstrated growth inhibition on *P. vulgaris* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml with the respective mean zones of inhibition of 2±0.20, 3±0.20, 7±0.10 and 8±0.10. There were mean zones of inhibition of 7±0.10 and 8±0.10 exhibited by this fraction on *E. coli* at the

respective concentrations of 5,000 and 10,000 µg/ml, whereas at lower concentrations of 1,000 and 2,000 µg/ml there were no growth inhibition. Also, this fraction showed no growth inhibitory effect on *K. pneumoniae* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. For the distilled H_2O-EtOAc interface fraction of the stem of the plant, there were growth inhibitory effects on *P. vulgaris* and *E. coli*. It recorded mean zones of inhibition of 6±0.10 and 7±0.30 on *P. vulgaris* at the respective concentrations of 5,000 and 10,000 µg/ml, whereas at lower concentrations of 1,000 and 2,000 µg/ml there were no growth inhibition. Also, this fraction recorded mean zones of inhibition of 5.5±0.42, 7±0.44 and 8±0.52 on *E. coli* at the respective concentrations of 2,000, 5,000 and 10,000 µg/ml. At a lower concentration of 1,000 µg/ml there was no growth inhibition. This interface fraction demonstrated no growth inhibitory effect on *K. pneumoniae* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The same results of no growth inhibition were recorded for the $CHCl_3$ and EtOAc fractions of the stem of the plant on the three clinical isolates just as for the same fractions on *S. aureus* and *P. aeruginosa* as shown in Table 5a.

The results recorded in Table 5b are indications that the EtOH extract, the distilled H_2O and the distilled H_2O-EtOAc interface fractions of the stem of the plant contained toxic components which inhibited the growth of *E. coli* and *P. vulgaris*. This research finding is in support of the ethno-medicinal uses of *Maytenus senegalensis*.

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