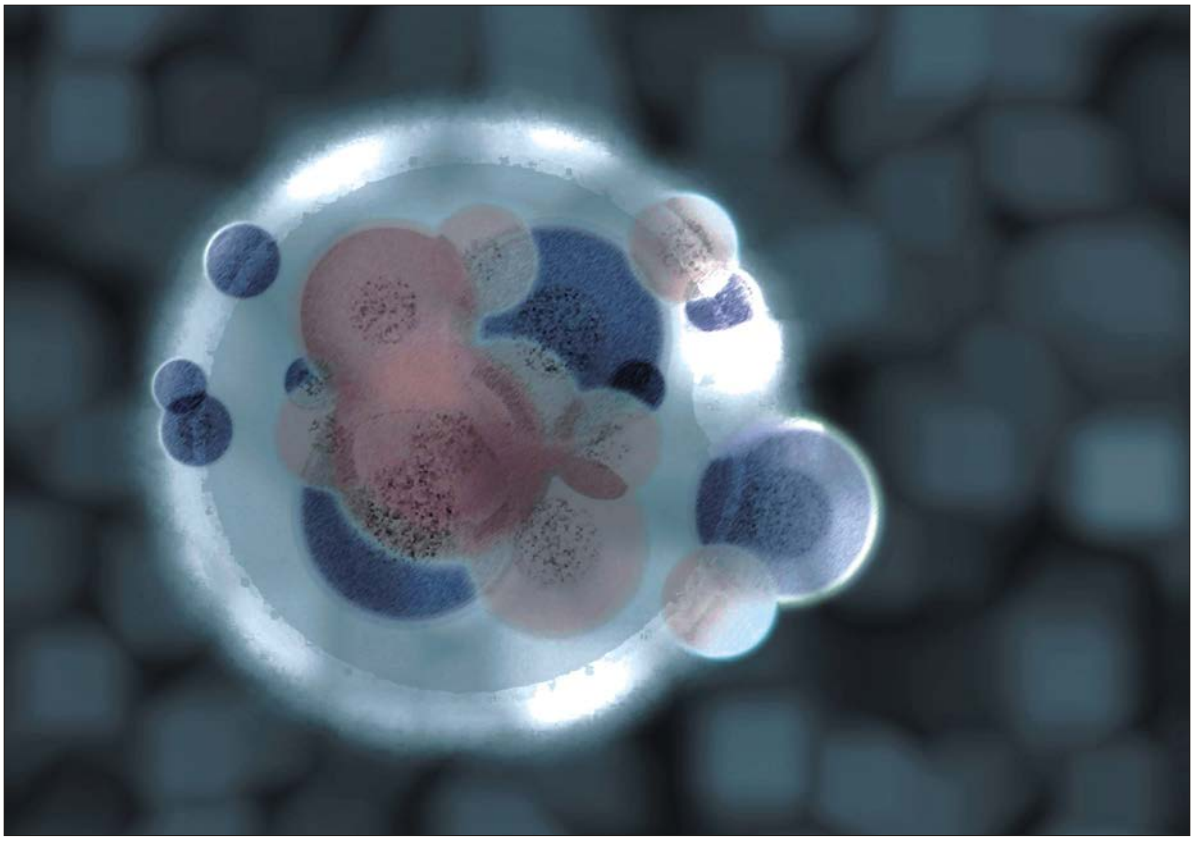

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In vitro Antimicrobial Activity of Extracts from Some Cameroonian Medicinal Plants

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Abstract: In order to valorise the pharmaceutical potential of natural products, 10 plants of nine different families collected on ethnobotanic informations around Yaoundé in Cameroon were extracted. Seventeen organic and hydro-organic extracts were tested for their biological effects. The *in vitro* evaluation of antibacterial and antifungal activity was carried out by the well agar diffusion method on seven Gram positive and Gram negative bacteria (*P. aeruginosa*, *E. coli*, *S. faecalis*, *S. choelera*, *P. mirabilis* and *M. morganii*) and two groups of fungi (Filamentous, Yeast). The results showed that 12 of the 17 extracts demonstrated antibacterial activity against the seven pathogenic bacteria tested. The growth inhibition halos were ranged from 8.00 to 32.33. Among them, extracts of *Solanum aculeastrum* (Solanaceae) and *Syzygium guinensis* (Myrtaceae) showed the higher antibacterial activity. For the antifungal activity, growth inhibition halos varied from 8.00 to 17.55 mostly against *Geotrichum candidum* and *Penicillium* species. The extracts *S. aculeastrum* demonstrated antibacterial and antifungal activity.

Key words: Antimicrobial activity, growth inhibition, pharmaceutical potential

INTRODUCTION

Traditional medicine practice has been around for centuries and still remains very common in the developing world. It is estimated that about 80% or more of the world's population rely primarily on traditional medicine for their healthcare (Kurt *et al*., 2000). Plant extracts as well as other alternative forms of medical treatment have made large contributions to human health and well-being (Chukwujekwu *et al*., 2005). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996; Iwu *et al*., 1999). The use of medicinal plants in Cameroon has been demonstrated (Table 1) in the centuries (Betti, 2004).

In recent years, secondary plant metabolites (phytochemicals and phytotherapy), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin, 1985). The aim of this study was to evaluate the *in vitro* antimicrobial activity of extracts from 12 plants against several Gram-positive and Gram-negative bacterial as well as fungi isolates.

MATERIALS AND METHODS

Extract preparation: Plant materials dried at room temperature (30°C±2) were ground to fine powder. Each powder (250 g) was separately macerated by mixing in 1000 mL organic or a mixture of hydro-organic 4:1 (v/v) 72 h. Each resulting solution was filtered using Whatman filter paper No. 1, concentrated with rotavapor following in an air circulating oven at 54°C until total dryness. The experiment was repeated twice with the samepowder and the crude extract obtained was stored at 5°C. Each extract was suspended in the DMSO for the antibacterial and antifungal assay.

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Table 1: Ethnobotanic information of some plants traditionally used in Cameroon and some area in Africa

Species	Family	Vernacular name	Part of plants	Traditional used of plant
Acanthus montanus (Nees) T. Anders	Acanthaceae	Fondzap (Bamoun); Ngick (Baasa); Ndole	St-Le	Spain, gonorrhea, dysmenorrhoea, chronic ulcer, intestinal helminthiasis and pharyngitis.
Alanblackia florinbunda Oliv	Guttiferae	Bar bar (Lamso)	St-Ba	Cough
Centella asiatica (Liun) Urban	Apiaceae	Longio Diep (Bamena)	Le	Pharyngitis, dysmenorrhoea
Desmodium adscendeus (SW) DC	Leguminosea-Papilionoideae	Pepeur (Bakossi); Owondo bekone (Bulu)	Pt-Le	Dysenteria, abdominal pain, haemorrhoids, urinal infections and gonocorrhea
Euphorbia hirta L.	Euphorbiaceae	Okoul bifes (Ewondo)	Pt-Le,	Asthma, respiratory tract inflammations, coughs, chronic bronchitis and other pulmonary disorders. diarrhoea, dysentery, ulcerated oral mucosa, polyhydramnios, chest pain, pneumonia, diarrhoea, schizophrenia and haemorrhoids
Lophira alata Banks. ex Gaertn. f.	Ochnaceae	Okoga (Ewondo)	Ba	Toothache, inflammatory diseases and analgesic
Senna alata (L.) Roxb and	Leguminoseae-Ceasalpiniaceae	Ngom (Ewondo) Seu nansa (Bamoun)	Le-St-	Haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, bleunorrhagia, skin infections, syphilis, diabetes, gastroenteritis, jaundice, Eczema, Thyphoenteritis, Ringwound food poison
Solanum aculeastrum	Solanaceae	Sircerka (Bana) breast cancer,	Fr	Jigger wounds, gonorrhea, cancer, particularly Dunal subsp constipation, tubal blockage, gastritis and epilepsy
Syzyguim guineuses DC.	Myrtaceae			Antitussive, coughs Pelvic inflammatory disease
Leea guianeusis Royen ex L.	Leeaceae	Totoun (Bassa)	Le	Spleen in children, edematogenic pelvic inflammatory
Morinda lucida Benth	Rubiceae	Aken (Ewondo)	Le	Fever, abdominal pain, dysenteria and splenomegaly

st-ba : Bark of stem; Le: leaf; Pt: Whole plant; Fr: Fruit; Be: Berries

Micro-organisms: The micro-organisms used for the antibacterial evaluation are clinical isolates and provided by the Centre Pasteur of Cameroon of Yaoundé. These include *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Schigella cholera*, *Proteus mirabilis* and *Morganella morganii*. *Filamentous fungi* (*Fusarium* sp., *Penicilium* sp., *Helmintosporum* sp. and *Aspergilus flavus*) and yeast (*Candida albicans*, *Candida kefyr* and *Geotrichum candidum*. The microbes used for antifungal activity were isolated from clinical specimens and identified in the Mycology laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I.

Antibacterial assay: The antimicrobial assay was performed by well agar diffusion method (Parekh and Chanda, 2007). The molten Mueller Hinton agar was inoculated with 100 μL of the inoculum (1×10^8 cfu mL^{-1}) and poured into the Petri plate. For agar well diffusion method, a well was prepared in the plates with the help of a corkborer (0.6 cm). Fifty microliter of the test extract (50 mg mL^{-1}) was introduced into the well. The plates were incubated overnight at 37°C for 18 h. Microbial growth was determined by measuring the diameter of the inhibition zone. The pure solvents were used instead of the extract as the negative control while the gentamicine (0.5 mg mL^{-1}) was utilised for the positive control. The result was obtained by measuring the diameter of the inhibition zone with a caliper-square. The experiment was done three times and the mean values are presented.

Antifungal assay: Cultures of filamentous fungi and yeast were treated with different extracts (50 mg mL^{-1}) as previously described in the antibacterial assay. Ketoconazole was used as a positive control at the concentration of 2 mg mL^{-1}. Following incubation at 28°C for 48 h, the diameters (mm) of the growth inhibition halos were measured using a caliper-square.

Statistical analysis: The results were expressed as mean±SD (mm). Data were analysed by one way analysis of variance (ANOVA) followed by student-Newman-Kerls test ($p<0.05$).

RESULTS AND DISCUSSION

The antibacterial activity of 17 extracts of plant species were assayed *in vitro* by agar well diffusion method against 7 bacteria species and fungi isolates.

A total of seventeen and eleven extracts of plants are tested, respectively, for their *in vitro* antibacterial and antifungal activities (Table 2, 3). The diameter of inhibition of the extracts varied from 8.00 to 32.33 mm. The effects of the extracts on seven bacteria growth showed that 11 (64.7%) extracts produced significant antibacterial activity. Six (35.29%) extracts were active on more than three of seven bacteria used in the experiment. Most of the bacteria tested were less sensitive to CH_2Cl_2 and MeOH extracts of *A. montanus* and *L. guineensis* respectively. For the antifungal activity, only 5 (45.5%) of eleven extracts showed no antifungal activity. Only EtOH extract of *S. aculeanstrum* demonstrated a weak inhibition growth of five fungal species.

Table 2: Antibacterial activity of extracts

		Diameter of inhibition (mm)						
Species	Extract	*S. aurens*	*P. aeruginosa*	*E. coli*	*S. faecalis*	*M. morganii*	*P. mirabilis*	*S. cholera*
A. montauns	A	12.00±1.73	Ø	20.33±1.52	9.33±1.15	Ø	Ø	11.33±1.55
A. florinbunda	A	16.00±2.00	18.67±2.08	19.33±1.15	14.50±0.70	20.33±1.55	12.67±0.57	12.33±0.57
C. asiatica	A	11.67±0.57	ND	16.00±2.00	23.33±3.05	Ø	Ø	12.67±2.08
	D	13.33±1.52	11.67±0.57	11.00±1.00	22.00±1.00	10.33±2.08	14.00±1.00	23.33±2.08
D. adscendens	B	Ø	10.00±0.00	ND	17.00±3.00	ND	10.50±2.12	16.50±0.70
	C	13.33±1.15	13.00±1.00	17.00±1.00	19.00±1.00	17.67±3.21	12.67±0.57	15.00±1.00
E. hirta	A	13.33±3.21	Ø	ND	12.00±0.00	8.00±1.00	Ø	9.67±0.57
	B	23.33±7.23	18.67±2.08	24.33±1.15	26.00±3.46	13.00±1.00	16.33±4.16	14.33±2.51
L. alata	B	15.67±3.21	15.33±3.21	13.67±2.51	21.33±5.77	17.50±0.70	12.67±1.52	11.00±1.00
L. guianensis	B	8.00±1.00	11.67±1.52	Ø	11.00±2.00	Ø	ND	ND
M. lucida	B	14.33±1.52	15.33±0.57	12.33±2.08	13.00±1.00	12.33±0.57	11.00±1.73	15.33±0.57
S. alata	E	13.33±2.30	16.33±1.15	18.67±0.57	22.67±1.52	13.67±1.52	12.00±1.00	18.67±1.52
S. aculeastrum	B	28.33±2.08	32.33±3.51	25.33±1.52	28.00±2.00	11.00±1.00	29.67±1.15	14.33±0.57
	E	19.33±3.51	28.67±3.05	27.67±1.52	25.67±0.57	8.67±0.57	29.67±1.52	23.67±0.57
S. guinensis	B	14.33±2.08	16.67±0.57	20.67±1.52	20.33±1.52	12.67±0.57	13.33±2.30	10.67±2.08
	E	14.33±1.52	21.00±2.00	22.33±2.50	21.33±3.21	17.67±2.30	16.67±1.52	14.00±1.00
L. guianensis	C	13.00±1.73	13.67±1.52	13.00±1.00	19.00±1.00	12.67±1.15	12.67±1.52	10.67±1.52
Gentamicine		31.00±3.00	35.67±2.08	38.67±1.52	36.33±2.51	27.67±1.52	32.33±2.51	30.33±0.57

N.B: A: Methylene chloride extract; B: Methanol extract; C: Hydro-methanol extract; D: Hydro-methanol extract; E: Ethanol extract; ø: Diameter of inhibition > 8 mm; ND: Non-Determined; * Tested twice, all values given are the mean±SD (mm)

Table 3: Antifungal activity of plant extracts

Plants species	Extracts tested	*C. albicans*	*C. kefyr*	*G. caudidum*	*M. furfux*	*F. specie*	*P. specie*	*H. specie*	*A. flavus*
D. adscendens	C	0	0	9.67±0.57	0	0	10.00±1.00	11.67±0.57	0
C. asiatica	D	0	0	9.00±1.00	0	0	15.00±1.00	0	0
E. hirta	B	ND	ND	12.33±0.57	ND	ND	ND	ND	ND
L. guineensis	C	0	0	0	0	0	0	0	0
S. guineensis	B	0	0	0	0	0	0	0	0
S. *aculeastrum*	B	10.00±1.00	13.00±1.00	13.33±0.57	0	0	15.00±1.00	11.67±0.57	0
S. alata	D	0	0	14.00±1.00	0	0	0	0	0
A. florinbunda	A	0	0	0	0	0	0	0	0
L. alata	B	11±2	0	11±1	0	0	0	0	0
M. lucida	B	8.33±0.570	0	0	0	0	14.33±1.15	0	0
S. guineensis	E	0	0	0	0	0	0	0	0
Ketoconazole		18.00±2.00	0	18.67±1.52	19.67±1.52	16.33±1.55	26.67±1.52	0	0

N.B: A: Methylene chloride extract; B: Methanol extract; C: Hydro-methanol extract; D: Hydro-methanol extract; E: Ethanol extract; 0: Diameter of inhibition > 8 mm; ND: Non-Determined; * Tested twice, all values given are the mean±SD (mm)

The remaining extracts 5 (45.5%) showed an inhibition halo on one or two fungi species. With respect of the distribution of the biologically active constituents present in the extracts, results indicated that the polar extracts are more effective than the non polar ones. According to the growth inhibition halos of the extracts, the results were interpreted as follow: no activity (<7 mm halo), weak activity (7-10 mm halo), moderate activity (11-16 mm halo) and high activity (>16 mm halo) (Monks *et al*., 2002).

The seventeen extracts of eleven medicinal plants selected were tested for the *in vitro* antimicrobial activity by assessing their ability to inhibit bacteria and fungi growth (Table 2, 3). Regarding the potency of antibacterial activity of these extracts at the concentration tested, these extracts could be separated into three groups.

The first group includes six (35.29%) extracts showed the higher inhibition growth on more than three antibacterial. It concerns *S. aculeanstrum* (MeOH and EtOH extracts), *S. guineensis* (EtOH extract), *S. alata* (aqueous-EtOH extract), *E. hirta* (MeOH extract) and *A. florinbunda* (CH_2Cl_2 extract). All these plants are used for the treatment of various diseases in Africa (Adjanahoun *et al*., 1991; Igoli *et al*., 2005). The results in this study demonstrated that the MeOH extract of *S. aculeanstrum* is the most active. *S. aculeastrum* is widely used in traditional medicine for the treatment of human and livestock diseases (Hutchings *et al*., 1996), jigger wounds and gonorrhea (Agnew and Agnew, 1994). The berries and leaves of *S. aculeastrum* demonstrated antiproliferative on three human tumour cell lines HT29, Hela and CMF7 (Koduru *et al*., 2006c). The antimicrobial and antioxidant activities of *S. aculeastrum* have been demonstrated by Koduru *et al*. (2006a). The oil from *S. aculeanstrum* revealed the presence of terpenoids, alkanes, aldehydes, ketones, fatty acids and esters, diterpenes, aromatic hydrocarbons and miscellaneous compounds (Koduru *et al*., 2006b). Alkaloids, steroids and terpenoids have been reported to have good activities (Cowan, 1999).

In this study both MeOH and EtOH extracts of *S. aculeastrum* demonstrated significant inhibition growth of all bacteria tested except *M. morganii*. The aqueous-EtOH extract *S. alata* exhibited moderate antibacterial and antifungi (*C. albicans*, *G. candidum*). Traditional healers have been used the leaves of *Senna alata* (L.) Roxb. for a long time for the treatment of tinea versicolor and ringworm infection and other diseases in Africa (Kochar, 1981; Adjanahoun *et al*., 1991; Igoli *et al*., 2005; Wuthi-Udomlert *et al*., 2005). Its EtOH extract was reported to inhibit *D. congolensis* growth (Ali-Emmanuel *et al*., 2003). Makinde *et al*. (2007) in contrary showed higher antifungal activity of aqueous-MeOH extract of *S. alata* on fungi (*Microsporum canis*, *Blastomyces dermatitidis*, *Trichophyton mentagrophyte*, *Candida albicans*, *Aspergillus flavus*) and weak inhibition activity on bacteria (*Dermatophilus congolensis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Corynebacterium parvum*, *Actinomyces bovis*, *Nocardia asteroids*, *Clostridium septicum* and *Bacillus pumilus*). Weak antifungal activity of aqueous-EtOH, HCl, EtOH, lyophilized and aqueous extracts of leaves of *S. alata* on dermatophyte and C. albicans have been demonstrated (Wuthi-Udomlert *et al*., 2005).

The screening of bioactive molecules of leaves of *S. alata* revealed the presence of anthraquin one aglycone, anthraquinone glycosides (Wuthi-Udomlert *et al*., 2005), phenolics and terpenoids, alkaloid salt, alkaloid (Makinde *et al*., 2007). Study results demonstrated that the MeOH extract of *E. hirta* is more active to all bacteria than the CH_2Cl_2 ones confirming that polar extract is more active. Similar results were obtained with the EtOH extract of leaves of *E. hirta* on *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* at the concentration of 200 mg mL^{-1} (Ogbulie *et al*., 2007). The antiplasmodial activity of CH_2Cl_2 and EtOH extracts of *E. hirta* have been reported Tona *et al*. (1999). Phytochemical studies of *E. hirta* reported in the literature showed the presence of terpenes, saponins, alkaloids, steroids and cardiac glycosides (Oliver-Bever, 1986; Parekh and Chanda, 2007). Both MeOH extracts of *S. guineensis* and *A. florinbunda* demonstrated weak antibacterial on *S. cholera*. *A. florinbunda* is commonly by the population of Baka pygmies in Cameroon and Central Republic Africa for the treatment of cough (Betti, 2004).

The second group of five (29.45 %) extracts included those which showed moderate antibacterial activity on at least 4 bacteria tested. They included MeOH extracts of *S. guineensis* and *M. lucida* and *L. alata* aqueous-EtOH extracts of *L. guineensis*, *D. adscendens* and aqueous-MeOH extract of *C. asiatica*. This results showed that *Monrinda lucida* Benth possess antibacterial property. This plant is used in tropical Africa for their therapeutic value in the treatment of antiparasitic diseases (Kambu, 1990; Tona *et al*., 1999). Some biological properties of leaves of *M. lucida* such as Schistosomicidal (Adewunmi and Adesogan, 1986a), Mollucidal (Adewunmi and Adesogan, 1986b) and trypanosomicidal (Asuzu and Chineme, 1990) have been reported. Adomi (2006) demonstrated that the aqueous bark of *M. lucida* was sensitive only on *S. aureus* and *P. aeruginosa* while its ethanol extract showed the great inhibition halos on *S. aureus*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *B. subtilis*, *Flavobacterium* sp. and *Centella asiatica* has been used in traditional medicine in Asia for hundreds of years as a fresh eaten vegetable (Hamid *et al*., 2002).

This plant has been effectively used in folk for the treatment of inflammation, anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly (Duke, 2002). It is also believed, to have beneficial effects in improving memory and treating mental fatigue, anxiety and eczema (Duke, 2002). In this study *C. asiatica* demonstrated potential antimicrobial activity. This property is attributed to triterpene, saponins, asiaticoside, sapogenin asiatic acid, madecassoside and madecassic acid major constituents isolated from the plant and which the antibacterial activity has been demonstrated by Cowan (1999) and Somchit *et al*. (2004).

The aqueous extract of *C. asiatica* possesses antioxidant, cognitive-enhancing, antiepileptic, antinociceptive and anti-inflammatory properties (Gupta *et al*., 2003; Somchit *et al*., 2004). An aqueous-EtOH extract of *L. guineensis* showed no antifungal activity. *S. faecalis* is found to be the most sensitive among seven bacteria tested. The chemical composition of aqueous extract of *L. guineensis* reported in the literature indicated the presence of alkaloids, saponins and reducing sugars (Falodun *et al*., 2007). In this antifungal screening, an aqueous-EtOH extract of *D. adscendens*, *M. lucida* as well as *C. asiatica* showed poor inhibition on *Penicilium* sp. Several studies reported the antibacterial (Chukwujekwu *et al*., 2005; Ogbulie *et al*., 2007), anti-inflammatory and antimalarial (Chukwujekwu *et al*., 2005) activities of extract of *M. lucida*. A bark extract of a tree of *Lophira alata* is used as a folk medicine in western Africa for inflammations and ananalgesic activity. Three monoesters related to lophirosides and 4 lophirosides were isolated from *Lophira alata* (Tih *et al*., 1994).

The last group of these molecules exhibited weak anti-bacterial activity against *Micrococcus luteus* but do not act as defensive substances against insects and microorganisms (Fleming, 1999).

The last group of five (29.41%) including CH_2Cl_2 extract of *A. montanus*, *C. asiatica*, *E. hirta*, MeOH extract of *L. guineensis* and *D. adscendens*. All these extracts exhibited moderate inhibition the growth of 29.24% of bacteria tested. Poor antifungal activity of MeOH extract of *E. hirta* was noted in this study. The leaf decoction of *Acanthus montanus* (Nees) T. Anders (Acanthaceae) is used by Nigeria healers to treat chesty coughs (Obute, 2006). In general, all bacteria used in this study involved diseases such as urogenital and respiratory tract infection, chancre, nosocomial pathogens, diarrhoea and opportunistic infections (Atlas, 1988). The observed antibacterial properties corroborate its use in traditional medicine. The relatively high zone of inhibition exhibited by the extracts against *E. coli* is also of significance, since *E. coli* and *S. cholera* is a common cause of diarrhoea in developing countries. The large zones of inhibition exhibited by the extract against *S. aureus* and *P. aeruginosa* justified their use by traditional medical practitioners in the treatment of sores, bores and open wounds. *S. aureus* and *P. aeruginosa* have been implicated in cases of boils, sores and wounds (Ogbulie *et al*., 2007). The inability of some extracts to inhibit *M. morganii* and *P. mirabilis* may be that these bacteria to posses mechanisms by which they convert substances that inhibit their growth to non-toxic com-pounds.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999). Continued, further exploration of plant-derived antimicrobials is needed today.

The present study, of *in vitro* antimicrobial evaluation of some plants, forms a primary platform for further phytochemical and pharmacological studies. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds. In conclusion, EtOH extract of *S. aculeastrum* possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. Its MeOH extract showed weak antifungal property. Further research will be carried out on the fractionation of these interesting extracts in order to identify the active.

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