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Full Length Research Paper

Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L)

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Antimicrobial properties of leaf extracts of Senna obtusifolia (L) were investigated against both clinical and laboratory isolates of both bacteria and fungi using the disc diffusion method. Acetone extracts (12 mm zone diameter of inhibition, MIC 200 g/mL and MBC 300 g/mL) demonstrated the highest activity, followed by dichloromethane (8 mm zone diameter of inhibition, MIC 300 g/mL and MBC 400 g/mL), methane (7 mm zone diameter of inhibition, MIC 400 g/mL and MBC 400 g/mL) and hexane (6 mm zone diameter of inhibition, MIC 800 g/mL and MBC 1000 g/mL). Water extracts demonstrated the least activity against the test bacteria and fungi (4 mm zone diameter of inhibition, MIC 800 g/mL and MBC 800 g/mL). Phytotoconstituents present included Saponins, Tannins, Alkaloids and Flavonoids. S. obtusifolia (L) can be used to source antibiotic substances for possible treatment of bacterial and fungal infections including gonorrhea, pneumonia, urinary tract and some mycotic infections.

Key words: *Senna obtusifolia* (L), antimicrobial property, extract antibiotic.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi et al., 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000).

Long before mankind discovered the existence of microbes, the idea that certain plants had healing poten-tial, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accep-ted. Since antiquity, man has used plants to treat com-mon infectious diseases and some of these traditional medicines are still included as part of the habitual treat-ment of various maladies. For example, the use of bear-berry (*Arctostaphylos uvaursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (R´ıo and Recio, 2005). Since the discovery of penicillin (1929) and its use in chemotherapy in 1941 as a response to the great fatalities in the Second World War, a great number of important antibiotics have been found (El-Bana, 2007). The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus (El astal et al., 2005). *Senna obtusifolia* (syn. *Cassia obtusifolia* L., *Cassia tora*, *Emelista tora*) (Fabaceae (alt. Leguminosae, subfamily Caesalpinioi-

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deae),commonly called Tasba (Hausa - Nigera), Tsetsa (Tiv - Nigeria), (ebisu-gusa (Japan), gyeolmyeongja (Korea), fedegoso (Portuguese), sinameki (Turkey) and sicklepod, coffepod, coffee weed or java bean (English) is a common weed of open disturbed areas (Hawaii) (Wagner et al., 1999), arid lowlands (Galapagos Islands) (McMullen, 1999) and disturbed areas such as drainage channels and overgrazed pastures in dense seawards along rivers and flood plains sea level to 1200 feet (Australia) (Smith, 2002). It grows wild in North, Central and South America, Asia and Africa and is considered a particularly serious weed in many places. The species name comes from the Latin obtus (dull or blunt), and folium (leaf). Its leaves, seeds and root are used medicinally, primarily in Asia. It is believed to possess laxative effect, as well as to be beneficial for the treatment of eye infections. The plant's seeds are a source of cassia gum, a food additive usually used as a thickener. As a folk remedy, the seeds are often roasted, then boiled in water to produce tea and used for the treatment of diarrhea, tremors and for dark brown urine (urinary tract infections) . Roasted and ground, the seeds have also been used as a substitute for coffee. The leaf decoction is used as febrifuge in Cöte d'Ivoire and Nigeria and for the treatment of scorpion stings, gingivitis, urinary tract infections, dysentery and diarrhea in Nigeria, as traditional fever remedies in Zimbabwe and for the treatment of cough in Tzeital Maya (David, 2002; Fowler, 2006). The toxic principles of the plant include anthraquinones, emodin glycosides, toxalbumins and alkaloids (Majorie, 1999). With increase in antibiotic resistance, cost and inaccessibility (especially in rural areas) to some orthodox modern antibiotics, traditional weeds are fast gaining popularity even to urban and civilized dwellers. In addition, considering the wide medicinal application of this plant, this work was set out in order to investigate the antimicrobial activity of leaf extracts of *S. obtusifolia* against some pathogenic bacteria and fungi and to ascertain the chemical constituents that may be present.

MATERIALS AND METHODS

Plant storage

S. obtusifolia (L.) were collected around the Department of Microbiology Complex of the Federal University of Technology, and were authenticated by Mr. D.F. Jatau of the Department of Forestry and Wildlife Management, School of Agriculture and Agricultural Technology, Federal University of Technology, Yola Adamawa State, Nigeria. The leaves were separated from stems, washed in clean water, and dried at room temperature (Eloff, 1998). The dried plants were milled to a fine powder in a Macsalab mill (Model 200 LAB), Eriez®, Bramley, and stored in the dark at room temperature in closed containers until required.

Extraction procedure

Dried plant leaves were extracted by weighing samples of 1 g of finely ground plant material and extracting with 10 mL of acetone, hexane, dichloromethane (DCM) or methanol (technical grade-Merck) and boiled water in polyester centrifuge tubes. Tubes were vigorously shaken for 3 to 5 min in a Labotec model 20.2 shaking machine at high speed. After centrifuging at 3500 rpm for 10 min the supernatant was decanted into pre-weighed, labeled containers. The process was repeated three times to exhaustively extract the plant material and the extracts were combined. The solvent was removed under a stream of air in a fume cupboard at room temperature and the extraction efficiency was quantified by determining the weight of each of the extracts (Gidado et al., 2005; Masoko and Eloff, 2005).

Antimicrobial activity screening

The antimicrobial activity of the crude extract was screened against four gram-negative bacteria; Neisseria gonorrheae, Salmonella sp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and two gram-positive bacteria; *Staphylococcus aureus* and *Streptococcus aerugenosa* (clinical isolates) obtained from the Microbiology Laboratory of the Specialist Hospital Yola, Adamawa State Nigeria; two standard laboratory isolates each of gram-negative bacteria *Escherichia coli* (EC 002 MBFTY) and *Salmonella typhi* (ST 008 MBFTY); and gram-positive bacteria *S. aureus* (SA012 MBFTY) and *Streptococcus pneumoniae* (SN 006 MBFTY); and four fungi, Aspergillus niger (AN 082 FFTY), Aspergillus tamari (FT 001 MFFTY), *Candida albicans* (CA 032 FFTY) and *Fusarium oxysporum* (FO 004 FFTY) (standard laboratory isolates), all obtained from the Microbiology Laboratory, Department of Microbiology, Federal University of Technology Yola, Adamawa State, Nigeria. The antimicrobial activity was determined by the paper disc diffusion method (Ayandele and Adebiyi, 2007) using Mueller-Hinton agar plates (MHA, oxoid) (for all the bacteria) and potato dextrose agar plates (PDA, oxoid) (for the fungi) previously inoculated with 18 h old Nutrient broth (NB, oxoid) culture (0.5 Macfarland Standard) for the bacteria or spores (10^6 spores/mL for the fungi) suspension in Potato Dextrose Broth (PDB, Oxoid) of the test organisms, respectively. Sterilized paper discs (6 mm), soaked in a known concentration of the crude extracts of *S. obtusifolia* (L.) (5000 g/mL per disc) in DMSO were applied over each of the culture plates previously seeded with the 0.5 McFarland (for bacteria) and 10^6 spores/mL (for fungi). Antibiotic discs of ofloxacin (30 g/m) was used as positive control for bacteria, clotrimazole (30 g) was used for fungi and sterilized paper discs without extracts or antibiotics were used as negative controls for both the bacteria and fungi. The experiment was performed in triplicate. Incubations were at 37^oC for 24 - 48 h for bacteria and *C. albicans* and at room temperature for 72 h for the other filamentous fungi. Following incubation the zones of inhibition formed were measured and the mean diameter obtained. Overall, cultured bacteria with halos equal to or greater than 7 mm and fungi with 10 mm halos were considered susceptible to the tested extract (Nascimento et al., 2000).

Preliminary phytochemical studies

The extracts were subjected to various phytochemical tests to determine the active constituents present in the crude aqueous and ethanoic extracts. The slightly modified method of Okerulu and Ani (2001) was used.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the crude extracts was also determined using the same method except that the paper discs were soaked in different concentrations of the crude extracts

Table 1. Phytochemical constituents of root extracts of Deuterium microcarpum.

Extract	pH	% extraction	Saponins	Tannins	Alkaloids	Flavonoids	Balsams	Anthraquinones
Water	5.3	52	-	-	+	+	-	-
Acetone	5.1	44	+	+	+	+	-	-
Dichloromethane	5.0	28	+	+	-	-	-	-
Hexan	5.5	50	-	-	+	+	-	-
Methanol	5.2	38	-	+	+	+	-	-

Key: - - = absent,; + = present.

dispersed in water (10 - 2000 µL). After incubating at 24 h at 37°C, the MIC of each sample was determined by measuring the optical density in the spectrophotometer (620 nm), and comparing the result with those of the non inoculated NB and PDB (Nascimento et al., 2000).

The minimum bactericidal concentration (MBC) of the plant extract on the clinical bacterial isolates was carried out according to National Committee for Clinical Laboratory Standard (1990) provision. Briefly, 1 ml was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and streaked on MHA (for bacteria) and PDA (for fungi) and incubated for 24 h (for bacteria) and 72 h (for fungi) . The least concentration of the extract with no visible growth after incubation was taken as the minimum bactericidal concentration.

Evaluation of the synergistic effect of antibiotics and plant extracts or phytochemicals on the test organisms

This evaluation was done according to Muroi and Kubo (1996). Aliquots of 100 µL of resistant bacterial cultures (0.5 MacFarland Standard) grown in 10 mL of nutrient broth for 6 h were inoculated in nutrient broth supplemented with the respective antibiotics (50 µg/mL) and 10^6 cells/mL fungal cultures grown in PDB supplemented with 100 g/mL coltrimazole with different concentrations of plant extracts. The concentration for plant extracts ranged from 10 to 500 µg/mL, based on MIC values that had previously been evaluated. The growth conditions were the same as previously mentioned. After 48 h, the optical density of each sample was documented and compared to those of MIC to verify any synergistic effect among the tested compounds.

RESULTS AND DISCUSSION

Percentage extraction for the different solvents used was 52% (water), 50% (hexane), 44% (acetone), 38% (methanol) and 28% (dichloromethane). Water is a universal solvent and is generally used in traditional settings to prepare the plant decoctions for health reme-dies. It has been reported that many natural products including pigments, enzymes and bioactive components are soluble in water, which explains the highest yield of extract, while some of the solvents especially acetone are selective for tannins (Majorie, 1999). All the extracts were acidic in nature (pH values ranging between 5.0-5.5). The acidity combined with bioactive components might enhance the antimicrobial activity of the extracts especially against the bacteria.

Qualitative phytochemical investigation revealed that the extracts contained some phytoconstituents. Saponins, tannins, alkaloids and flavonoids are present in the acetone extracts; tannins, alkaloids and flavonoids are found in the methanol extracts; alkaloids and flavonoids in water; and hexane extracts and saponins and tannins in dichloromethane extracts (Table 1). These bioactive components including thiocynate, nitrate, chloride and sulphates, beside other water soluble components which are naturally occurring in most plant materials, are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property to plants (Lutterodt et al., 1999; Pretorius et al., 2001; El astal et al., 2005). All the extracts demonstrated antimicrobial activity against both the test bacteria and fungi with the acetone extracts demonstrating the highest activity (12 mm zone diameter of inhibition), followed by the dichloromethane extracts (8 mm zone diameter of inhibition), while the water extracts demonstrated the least activity (2 mm zone diameter of inhibition) at 5000 g/mL (Figure 1). The water extracts did not demonstrate any reasonable activity against all the clinical isolates; *N. gonorrheae*, *Salmonella* sp, *P. aeruginosa*, *P. vulgaris*, *S. aureus* and *S. aerugenosa*. The acetone extracts were active against three of the clinical isolates; N. gonorrheae (6 mm zone diameter of inhibition), S. aureus (2 mm zone diameter of inhibition) and S. aerugenosa (8 mm zone diameter of inhibition), and all of the laboratory isolates; *E. coli* (10 mm zone diameter of inhibition) *S. typhi* (12 mm zone diameter of inhibition), *S. aureus* (6 mm zone diameter of inhibition), *S. pneumoniae* (4 mm zone diameter of inhibition), A. niger (4 mm zone diameter of inhibition), *A. tamari* (6 mm zone diameter of inhibition), *C. albicans* (8 mm zone diameter of inhibition) and *F. oxysporum* (6 mm zone diameter of inhibition). The dichloromethane extracts had activity against both the clinical (N. gonorrheae – 8 mm zone diameter of inhibition, *Salmonella* sp – 4 mm zone diameter of inhibition, *P. vulgaris* – 8 mm zone diameter of inhibition and *S. aerugenosa* – 4 mm zone diameter of inhibition) and laboratory isolates [E. coli – 10 mm zone diameter of inhibition), *S. aureus* - 8 mm zone diameter of inhibition), *S. pneumoniae* (4 mm zone diameter of inhibition), A. niger (4 mm zone diameter of inhibition), A. tamari (6 mm zone diameter of inhibi-

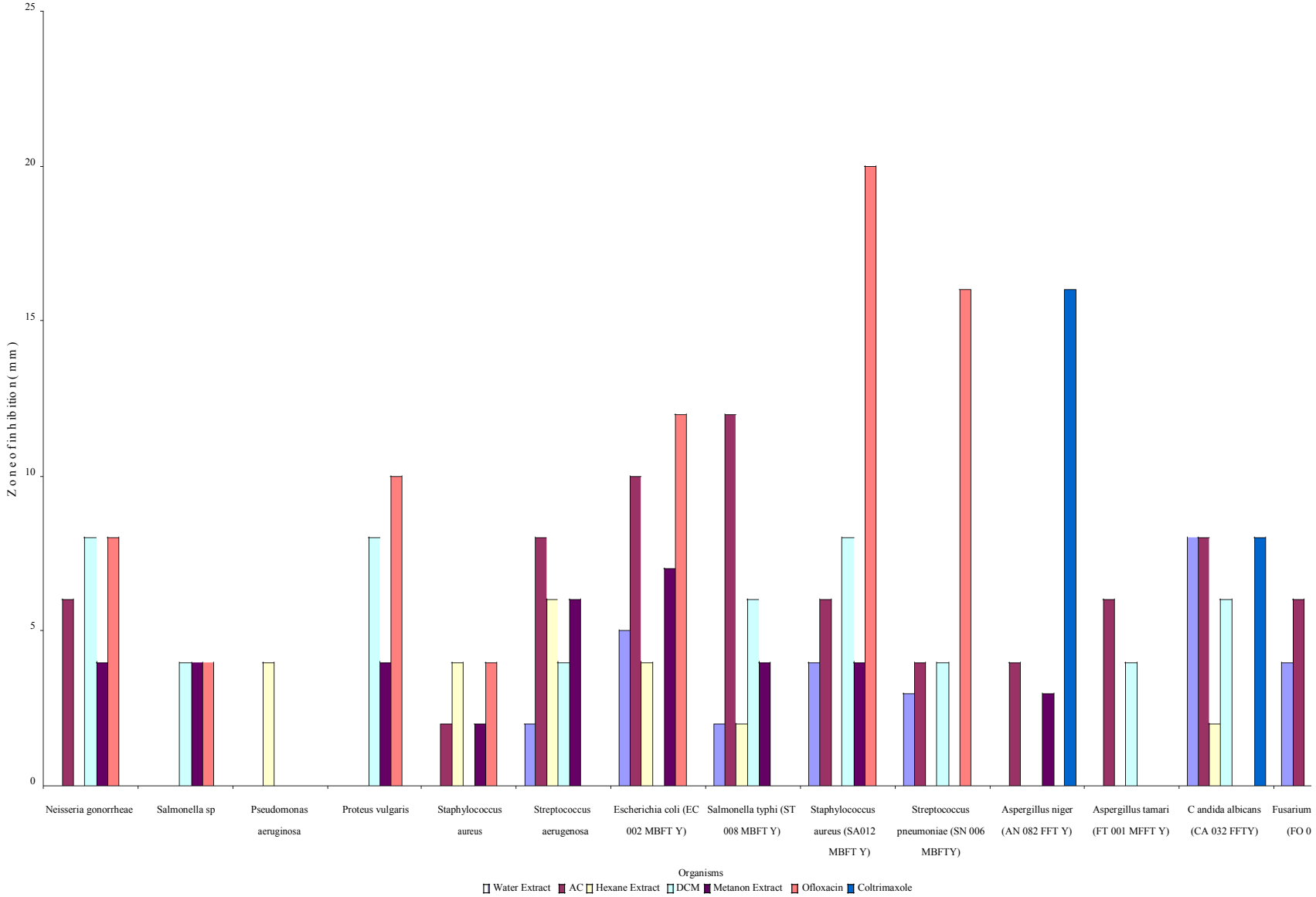

Figure 1. Antimicrobial activity of *Senna obtusifolia* (L) leaf extracts.

Table 2. Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of extracts of Senna otusifolia (L).

Organism	MIC (µg/ml)							MMC (µg/ml)						
	WE	**AC**	**HX**	**DCM**	**ME**	**Ofx (30 g)**	**col (30 g)**	**WE**	**AC**	**HX**	**DCM**	**ME**	**Ofx (30 g)**	Col (30 g)
Neisseria gonorrheae	2000	800	2000	400	1000	300	x	2000	1000	2000	400	1000	300	x
Salmonella sp	2000	1000	2000	1500	1500	800	x	2000	1000	1500	1500	1500	1000	x
Pseudomonas aeruginosa	2000	1000	1500	200	2000	1000	x	2000	1500	1500	2000	2000	1000	x
Proteus vulgaris	2000	1500	2000	300	1500	200	x	2000	1500	2000	400	1500	300	x
Staphylococcus aureus	2000	1500	1000	2000	2000	1000	x	2000	1500	1500	2000	2000	1000	x
Streptococcus aerugenosa	2000	600	800	1000	400	1000	x	2000	800	1000	1000	400	1000	x
Escherichia coli (EC 002 BFTY)	800	200	1000	2000	400	80	x	800	300	1000	2000	400	100	x
Salmonella typhi (ST 008 BFTY)	1500	200	1500	600	800	1000	x	1500	300	1000	800	800	1000	x
Staphylococcus aureus (SA012 MBFTY	1000	600	2000	300	800	10	x	1000	600	2000	400	800	40	x
Streptococcus pneumoniae (SN 006 BFTY)	1000	1000	2000	1000	1000	40	x	1000	1000	2000	1000	1000	40	x
Aspergillus niger (AN 082 FFTY)	2000	800	2000	2000	1000	x	40	2000	1000	2000	2000	1000	x	80
Aspergillus tamari (FT 001 FFTY)	2000	600	2000	800	1000	x	1500	2000	800	2000	800	1000	x	1500
Candida albicans (CA 032 FFTY)	600	400	2000	400	1000	x	400	600	400	2000	400	1000	x	400
Fusarium oxysporum (FO 004 FFTY	1000	600	2000	2000	500	x	1000	1000	600	2000	2000	500	x	1000

Key: WE = water extract; AC = acetone extract; HX = Hexane extract; DCM = Dichloromethane extract; ME = methanol extract; Ofx = Ofloxacin; Col = Coltrimaxole; x = not determined.

bition)and *C. albicans* -(8 mm zone diameter of inhibition)] at 5000 g/ml (Figure 1). Ofloxacin and coltrima-zole demonstrated the highest activities against both bacteria and fungi, respectively.

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella pneumoniae* is the most important member of the *Klebsiella* genus of Entero-bacteriaceae and it is emerging as an important cause of neonatal nosocomial infection (Gupta et al., 1993). *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients (Black, 1996). Infection caused by *Salmonella typhimurium* is a serious public health problem in developing countries and represents a constant concern for the food industry (Mastroeni, 2002). Proteus mirabilis causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores (Cheesbrough, 2000; Parekh and Chanda, 2007). The demonstration of activity against both gram-negative and gram-positive bacteria and fungi is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against both

Table 3. Synergistic activity of extracts of Senna obtusifolia (L) (30 g/ml) with antibiotics (30 g/ml).

Organisations	Zone of inhibition (mm)					
	E	0	EO	E	C	EC
Neisseria gonorrheae	6	8	14	x	x	x
Salmonella sp	-	4	10	x	x	x
Pseudomonas aeruginosa	-	-	8	x	x	x
Proteus vulgaris	-	10	12	x	x	x
Staphylococcus aureus	2	4	6	x	x	x
Streptococcus aerugenosa	8	-	1 0	x	x	x
Escherichia coli (EC 002 MBFTY)	10	12	18	x	x	x
Salmonella typhi (ST 008 MBFTY)	12	-	14	x	x	x
Staphylococcus aureus (SA012 MBFTY)	6	20	20	x	x	x
Streptococcus pneumoniae (SN 006 MBFTY)	4	16	10	x	x	x
Aspergillus niger (AN 082 FFTY)	4	x	x	4	16	14
Aspergillus tamari (FT 001 MFFTY)	6	x	x	6	-	-
Candida albicans (CA 032 FFTY)	8	x	x	8	8	8
Fusarium oxysporum (FO 004 FFTY)	6	x	x	6	2	2

Key: E = Extracts only; O = Ofloxacin alone; EO = Extrcat/Ofloxacin; C = Coltrimaxole alone; EC = Extrcat/coltrimaxole, x = not determined.

clinical and laboratory isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms prevalent in hospital environments.

The MIC and MMC of the extracts ranged from 200-2000 g/mL, with the acetone extracts demonstrating the lowest values (MIC 200 g/mL: MBC 300 g/mL each) against *E. coli* (EC 002 MBFTY) and *S. typhi* (ST 008 MBFTY), followed by the dichloromethane extracts against *S. typhi* (ST 008 MBFTY) (MIC 600 g/mL, MBC 800 g/mL) and *S. aureus* (SA012 MBFTY) (MIC 300 g/mL, MBC 400 g/mL) (Table 2). Most of the MIC values were lower than the MBC values indicating that the extracts could be bactericidal in action. Low MIC and MBC values are also an indication of high efficacy. Lower MIC and MBC values (Table 2) and higher zones of inhibition (Figure 1) for acetone extracts connotes higher solubility of phytoconstituents in the acetone compared to the other solvents used.

Different solvents have various degrees of solubility for different phytoconstituents (Majorie, 1999). Table 3 shows the effect of combination of extracts and antimicrobial agents on the test organisms. Results revealed an increased activity of both ofloxacin (30 g/mL) and coltrimaxole (30 g/mL) in the presence of the extracts (30 g/mL). At 30 g/mL, both ofloxacin and the extracts had no effect on *P. aerugenosa* (clinical isolate), but when combined, there was a remarkable activity (8 mm zone diameter of inhibition). At 30 g/mL the activity of the extracts and ofloxacin against *E. coli* (EC 002 MBFTY) were 10 and 12 mm (zone diameter of inhibi-tion), respectively but this increased to 18 mm when the extracts and the antibiotics were combined. A similar trend was observed with extract-coltrimaxole combination against the test fungi. At 30 g/mL, the activity of the extracts alone against *A. niger* (AN 082 FFTY) was 4 mm (zone diameter of inhibition) and that of coltrimaxole was 14 mm (zone diameter of inhibition), but this activity increased to 16 mm when the extracts and co-trimaxole were combined. Synergistic effect of some phyto-constituents on antibiotics against some resistant isolates had earlier been reported (Nascimento et al., 2000).

Conclusion

Extracts of *S. obtusifolia* (L) in this study demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria and fungi. The broad-spec-trum antibacterial activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as a health remedy in folklore medicine. Bioactive sub-stances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonor-rhea, pneumonia, eye infections and mycotic infections. Isolation, identification and purification of these phyto-constituents and determination of their respective antimi-crobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

REFERENCES

Ayandele AA, Adebiyi AO (2007). The phytochemical analysis and antimicrobial screening of extracts of Olax subscorpioidea. Afr. J. Bio

technol. 6(7): 868-870.
Black JG (1996). Microbiology: Principles and Application. Prentice Hall, New York. p. 260.
Cheesbrough M (2000). Medical Laboratory Manual for Tropical Countries. Microbiology, Linacre House, Jordan Hill Oxford. p.260.
David GC (2002). Cognition and cultural transmission of Tzetal Maya medical plant knowledge. http: //www.wiu.edu/users/dge 101/disabs.html.
El astal ZY, Aera A, Aam A (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. Pak. J. Med. Sci. 21(2):187. www.pjms.com.pk
El-Banna NM (2007). Antifungal activity of Comamonas acidovorans isolated from water pond in south Jordan. Afr. J. Biotech. 6(19): 2216-2219.
Eloff JN (1998). Which extract should be used for the screening and isolation of antimicrobial compounds from plants. J. Ethnopharm. 60:1-8.
Fowler DG (2006). Traditional fever remedies: a list of Zambian plants. http://www.giftshealth.org/ritam/news/Traditional_Fever_remedies 1.pdf
Gidado A, Ameh DA, Atawodi SE (2005). Effect of Nauclea latifolia leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. African J. Biotech. 4(1): 91-93.
Gupta P, Murali P, Murali MV, Faridi MMA, Kaul PB, Ramachandran VC, Talwar V (1993). Clinical profile of Klebsiella septicaemia in neonates. Ind. J. Paediatr. 60: 565-572.
Lorke D (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54: 275-287.
Lutterodt GD, Ismail A, Basheer RH, Baharudin HM (1999). Antimicrobial effects of Psidium guajava extracts as one mechanism of its antidiarrhoeal action. Malay. J. Med. Sci. 6(2): 17-20.
Mastroeni P (2002). Immunity to systemic Salmonella infections. Curr. Mol. Med. 2: 393-406.
Majorie MC (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4): 564-582.
Masoko P, Eloff JN (2005). The diversity of antifungal compounds of six South African Terminalia species (Combretaceae) determined by bioautography. Afr. J. Biotech. 4 (12):1425-1431.
McMullen CK (1999). Flowering plants of the Galápagos. Comstock Pub. Assoc., Ithaca, N.Y. p.370.
Muroi H, Kubo I (1996). Antibacterial activity of anacardic acids and totarol, alone and in combination with methicillin, against methicillin-resistant Staphylococcus aureus. J. Appl. Bacteriol. 80: 387-394.
Nascimento GGF, Lacatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol. 31(4): 886-891.
National Committee for Clinical Laboratory Standard (1990). Methods for the antimicrobial susceptibility testing. In: Manual of Clinical Microbiology. Am. Soc. Microbiol. Washington, DC. 5th edition, pp. 1105-1125.
Okerulu IO, Ani CJ (2001). The phytochemical analysis and antibacterial screening of extracts of *Tetracarpidium conophorum*. J.Chem. Soc. Nig. 26(1): 223-228
Owolabi .J Omogbai EKI, Obasuyi O (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. Afr. J. Biotechnol. 6 (14): 882-85.
Parekh J, Chanda S (2007). In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. Afr. J. Microbiol. Res.1 (6): 92-99.
Pretorius CJ, Watt E (2001). Purification and identification of active components of Carpobrotus edulis L. J. Ethnopharmarcol. 76: 87-91.
R´ıos JL, Recio MC (2005). Medicinal plants and antimicrobial activity. J. Ethnopharm. 100: 80–84.
Smith NM (2002). Weeds of the wet/dry tropics of Australia - a field guide. Environment Centre, Northern Territory. p.112.
Wagner WL, Herbst DR, Sohmer SH (1999). Manual of the flowering plants of Hawaii. Revised edition. Bernice P. Bishop Museum special publication. University of Hawai‘i Press/Bishop Museum Press, Honolulu. 2: 1919..