

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 µg/mL and MBC 300 µg/mL) demonstrated the highest activity, followed by dichloromethane (8 mm zone diameter of inhibition, MIC 300 µg/mL and MBC 400 µg/mL), methane (7 mm zone diameter of inhibition, MIC 400 µg/mL and MBC 400 µg/mL) and hexane (6 mm zone diameter of inhibition, MIC 800 µg/mL and MBC 1000 µg/mL). Water extracts demonstrated the least activity against the test bacteria and fungi (4 mm zone diameter of inhibition, MIC 800 µg/mL and MBC 800 µg/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 gonorrhoea, 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 potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment 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 (Rio 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 ), Tseta (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 Côte 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 gonorrhoeae*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and two gram-positive bacteria; *Staphylococcus aureus* and *Streptococcus aeruginosa* (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 Adebisi, 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 µg/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 µg/m) was used as positive control for bacteria, clotrimazole (30 µg) 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°C 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 ethanolic 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



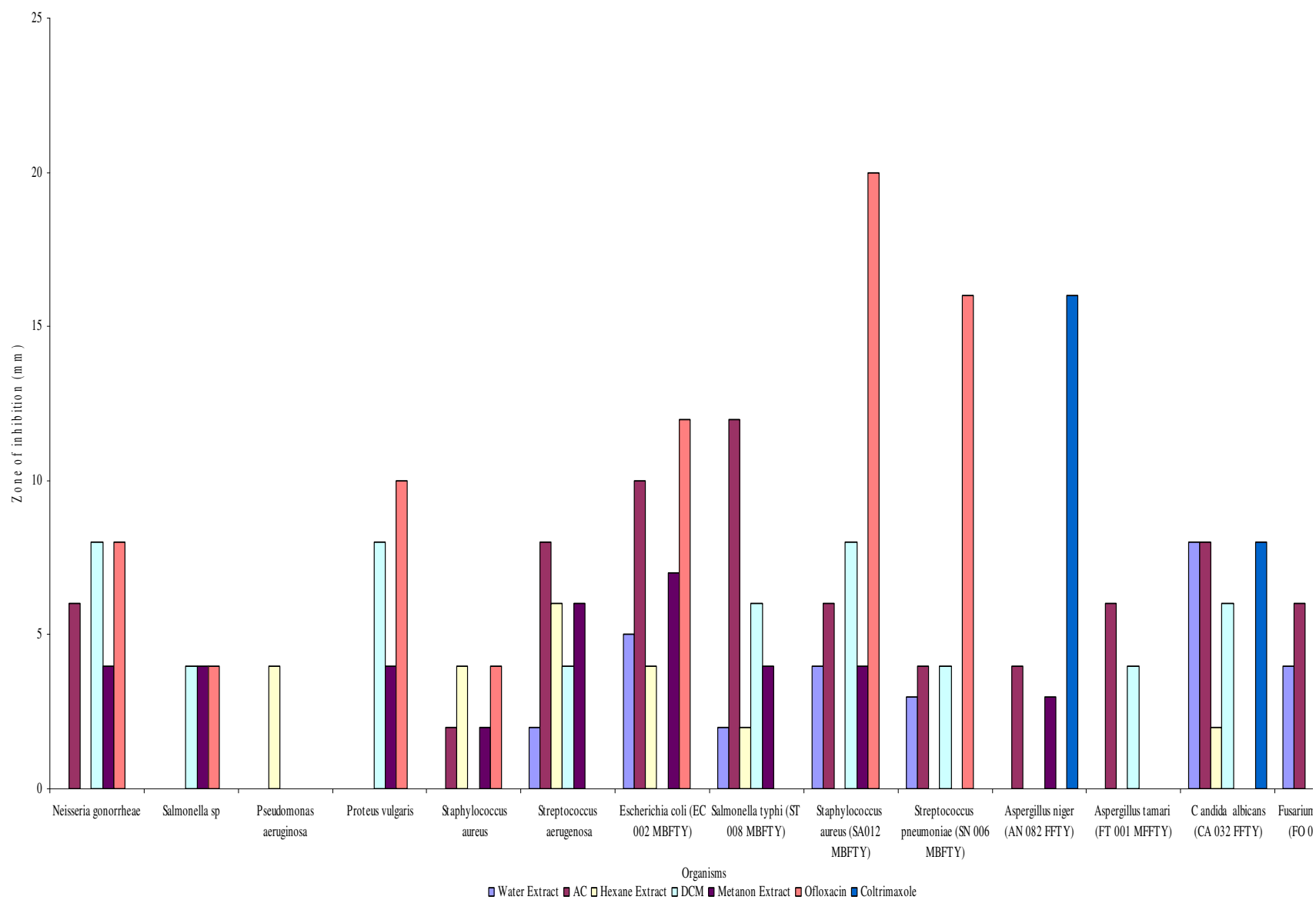


Figure 1. Antimicrobial activity of *Senna obtusifolia* (L) leaf extracts.

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

Organism	MIC ( $\mu\text{g/ml}$ )							MMC ( $\mu\text{g/ml}$ )						
	WE	AC	HX	DCM	ME	Ofx (30 $\mu\text{g}$ )	col (30 $\mu\text{g}$ )	WE	AC	HX	DCM	ME	Ofx (30 $\mu\text{g}$ )	Col (30 $\mu\text{g}$ )
<i>Neisseria gonorrhoeae</i>	2000	800	2000	400	1000	300	x	2000	1000	2000	400	1000	300	x
<i>Salmonella sp</i>	2000	1000	2000	1500	1500	800	x	2000	1000	1500	1500	1500	1000	x
<i>Pseudomonas aeruginosa</i>	2000	1000	1500	200	2000	1000	x	2000	1500	1500	2000	2000	1000	x
<i>Proteus vulgaris</i>	2000	1500	2000	300	1500	200	x	2000	1500	2000	400	1500	300	x
<i>Staphylococcus aureus</i>	2000	1500	1000	2000	2000	1000	x	2000	1500	1500	2000	2000	1000	x
<i>Streptococcus aeruginosa</i>	2000	600	800	1000	400	1000	x	2000	800	1000	1000	400	1000	x
<i>Escherichia coli</i> (EC 002 BFTY)	800	200	1000	2000	400	80	x	800	300	1000	2000	400	100	x
<i>Salmonella typhi</i> (ST 008 BFTY)	1500	200	1500	600	800	1000	x	1500	300	1000	800	800	1000	x
<i>Staphylococcus aureus</i> (SA012 MBFTY)	1000	600	2000	300	800	10	x	1000	600	2000	400	800	40	x
<i>Streptococcus pneumoniae</i> (SN 006 BFTY)	1000	1000	2000	1000	1000	40	x	1000	1000	2000	1000	1000	40	x
<i>Aspergillus niger</i> (AN 082 FFTY)	2000	800	2000	2000	1000	x	40	2000	1000	2000	2000	1000	x	80
<i>Aspergillus tamari</i> (FT 001 FFTY)	2000	600	2000	800	1000	x	1500	2000	800	2000	800	1000	x	1500
<i>Candida albicans</i> (CA 032 FFTY)	600	400	2000	400	1000	x	400	600	400	2000	400	1000	x	400
<i>Fusarium oxysporum</i> (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 = Coltrimazole; x = not determined.

bition) and *C. albicans* (-8 mm zone diameter of inhibition) at 5000  $\mu\text{g/ml}$  (Figure 1). Ofloxacin and coltrimazole 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 Enterobacteriaceae 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 µg/ml) with antibiotics (30 µg/ml).

Organisations	Zone of inhibition (mm)					
	E	O	EO	E	C	EC
<i>Neisseria gonorrhoeae</i>	6	8	14	x	x	x
<i>Salmonella</i> sp	-	4	10	x	x	x
<i>Pseudomonas aeruginosa</i>	-	-	8	x	x	x
<i>Proteus vulgaris</i>	-	10	12	x	x	x
<i>Staphylococcus aureus</i>	2	4	6	x	x	x
<i>Streptococcus aerugenosa</i>	8	-	10	x	x	x
<i>Escherichia coli</i> (EC 002 MBFTY)	10	12	18	x	x	x
<i>Salmonella typhi</i> (ST 008 MBFTY)	12	-	14	x	x	x
<i>Staphylococcus aureus</i> (SA012 MBFTY)	6	20	20	x	x	x
<i>Streptococcus pneumoniae</i> (SN 006 MBFTY)	4	16	10	x	x	x
<i>Aspergillus niger</i> (AN 082 FFTY)	4	x	x	4	16	14
<i>Aspergillus tamari</i> (FT 001 MFFTY)	6	x	x	6	-	-
<i>Candida albicans</i> (CA 032 FFTY)	8	x	x	8	8	8
<i>Fusarium oxysporum</i> (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 µg/mL, with the acetone extracts demonstrating the lowest values (MIC 200 µg/mL; MBC 300 µg/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 µg/mL, MBC 800 µg/mL) and *S. aureus* (SA012 MBFTY) (MIC 300 µg/mL, MBC 400 µg/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 connote 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 µg/mL) and coltrimaxole (30 µg/mL) in the presence of the extracts (30 µg/mL). At 30 µg/mL, both ofloxacin and the extracts had no effect on *P. aeruginosa* (clinical isolate), but when combined, there was a remarkable activity (8 mm zone diameter of inhibition). At 30 µg/mL the activity of the extracts and ofloxacin against *E. coli* (EC 002 MBFTY) were 10 and 12 mm (zone diameter of inhibition), 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 µg/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-spectrum antibacterial activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhoea, pneumonia, eye infections and mycotic infections. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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