Antibiotic Properties of Leaf Extracts of *Sennaalexandrina* (L)

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Abstract: The antibiotic properties of *Sennaalexandarina* (L.) leaves extracts were studied against five bacteria, two molds and yeast by using the disc diffusion method. Acetone extracts (12mm 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 mmzone 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 presentation included Saponins, Tannins, Alkaloids and Flavonoids. *S. obtusifolia*(L) can be used to source antibiotic substances.
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Key words: *Sennaalexandrina*(L), antibiotic property, extract antimicrobial.

1. Introduction

Herbs are a natural form of whole plants or their parts such as flower, root, oil, stems rich in bioactive chemical compounds so called "Herbiceuticals". (Rakesh Sharma2010). 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 medical 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.

*Senna*species are members of the family *Fabaceae*found worldwide (NPGS, 2008). The most important species are tinnevellysenna *(Sennaalexandrina*Mill.) currently used in various laxatives. Little is known about the agronomic characteristics of *Senna*species because they have primarily been considered as weeds. Several phytochemicals exist in *Senna with* potential to be used as human medicines. (M. Idu,,*et. al.,*2007& J. B. Morris. 2009)*C. acutifolia*, yielding the finest and most valuable variety of the drug is a small shrub about 2 feet high.

With the increase in antibiotic resistance, cost and inaccessibility (especially in rural areas) to some orthodox modern antibiotics, traditional weeds are fast gaining popularity even in urban and civilized dwellers. In addition, considering the wide medicinal application of this plant, The present paper focuses on antimicrobial activity of leaf extracts of *S. alexandarina* against some test bacteria and fungi and to ascertain the chemical constituents that may be present.

2. Material and Methods

Place of Plant collection &storage

S. alexandrina (L.) was collected from Alexandria in northern Egypt and transported to the Department of Microbiology. The leaves were separated from stems, washed in clean water, and dried at room temperature (Eloff, 1998). The dried leaves were milled to a fine powder in a Macsalab mill (Model 200LAB), 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 screening

The antimicrobial activity of the crude extract was screened against two gram-negative bacteria;

Esherichia coli (ATCC 14169) *Pseudomonas areuginosa* (ACCT 9027*)* and three gram-positivebacteria; *Micrococcusleutus* (ATCC9341), *Staphylococcus aureus*(ATCC 6538)and *Bacillus subtulis* (ATCC 6633),and two fungi, *Aspergillusniger.*(*ATCC 16404*), and *Fusariumoxysporum*(*ATCC 48112*)and one yeast *Candida albicans*(ATCC 10231), (standard laboratory isolates), all obtained from theNAMRO in Egypt. The antimicrobial activity was determined by the paper disc diffusion method (Ayandele and Adebiyi, 2007) using Mueller-Hinton agar plates (MHA, oxide) (for all the bacteria) and potato dextrose agar plates (PDA, oxide) (for the fungi) previously inoculated with 18 hold Nutrient broth (NB, oxide) culture (0.5 Macfarland Standard) for the bacteria or spores (106 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. alexandrina*(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 106 spores/mL (for fungi). Antibiotic discs of gatifloxacin (30 µg/ml) were used as positive control for bacteria, nastatin (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 37oC 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).

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 Ani2001) was used.

Determination of MIC

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 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). 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.

Table 1: Phytochemical constituents of leaf extracts of *Sennaalexandrina*.

Extract	pH	% extraction	Saponins	Tannins	Alkaloids	Flavonoids	Balsams	Anthraquinones
Water	5.4	50	-	-	+	+	-	-
Acetone	5.2	43	+	+	+	+	-	-
Dichloromethane	5.1	27	+	+	-	-	-	-
Hexan	5.6	48	-	-	+	+	-	-
Methanol	5.3	36	-	+	+	+	-	-

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

The synergistic effect of antibiotics and plant extracts on the test organisms

This evaluation was done according to (Muroi and Kubo 1996). Aliquots of 100 µL of resistant bacterial cultures (0.5 MacFarlandStandard) grown in 10 mL of nutrient broth for 6 h were inoculated in nutrient broth supplemented with the respective antibiotics (50 µg/mL) and 106 cells/mL fungal cultures grown in PDB supplemented with 100 µg/mL Nystatin with different concentrations of plant extracts. The concentration of 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.

3. Result and Discussion

The different solvents used have percentage extraction as 50%,48%,43%, 36%& 27% for water, hexane, acetone, methanol and dichloromethane respectively. Water is a universal solvent and is generally used in traditional settings to prepare the

plant decoctions for health remedies. It has been reported that many natural products including pigments, enzymes and bioactive components are soluble in water, which explains the high yield of the 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 5.1-5.6). The acidity combined with proactive components might enhance the antimicrobial activity of the extracts especially against the bacteria. Qualitative phytochemical investigation revealed that the extracts contained some Phyto-constituents. 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 compounds are known to be biologically active and therefore aid the antimicrobial activities of *the plant*. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with Proline rich protein (Igbinosa*et al*., 2009) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Igbinosa*et al.,* 2009). These observations therefore support the use of plantin herbal cure remedies.Li and Wang (2003) reviewed the bio-logical activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that planthas potential as a source of important bioactive molecules for the treatment and prevention of cancer.The presence of tannins in plantsupports the traditional medicinal use of this plant in the treatment of different ailments. The bioactive components including thiocynate, nitrate, chloride and sulfates, 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 *and wattl*., 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 µg/mL (Figure 1). The acetone extracts were active against all of the laboratory isolates; *E. coli* (10 mm zone diameter of inhibition), *S. aureus*(6 mm zone diameter of inhibition), *A. niger* (4 mm zone diameter of inhibition), *C. albicans*(8mm zone diameter of inhibition) and *F. oxysporum*(6 mm zone diameter of inhibition). The dichloromethane extracts had activity against laboratory isolates [*E. coli* – 10 mm zone diameter of inhibition), *S. aureus*- 8 mm zone diameter of inhibition), *A. niger* (4 mm zone diameter of inhibition), at 5000 µg/ml (Figure 1). Gatifloxacin and Nystatin 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.

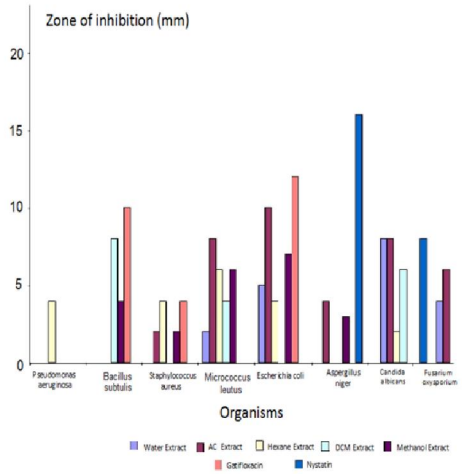

Figure (1): Antimicrobial activity of *Senna alexandrina* (L) leaf extract
AC: acetone
DCM : dichloromethane

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

Organism	MIC (g/ml)							MMC (g/ml)						
	WE	AC	HX	DCM	ME	Gfx	Nys.	WE	AC	HX	DCM	ME	Gfx	Nys.
Pseudomonas Aeruginosa (ACCT 9027)	2000	1000	1500	200	2000	1000	x	2000	1500	1500	2000	2000	1000	x
Bacillus subtulis (ATCC 6633)	2000	1500	2000	300	1500	200	x	2000	1500	2000	400	1500	300	x
Staphylococcus aureus(ATCC 6538)	2000	1500	1000	2000	2000	1000	x	2000	1500	1500	2000	2000	1000	x
Micrococcusleutus (ATCC9341)	2000	600	800	1000	400	1000	x	2000	800	1000	1000	400	1000	x
Escherichia coli (ATCC 14169)	800	200	1000	2000	400	80	x	800	300	1000	2000	400	100	x
Aspergillus niger	2000	800	2000	2000	1000	x	40	2000	1000	2000	2000	1000	x	80
(ATCC 16404)	600	400	2000	400	1000	x	400	600	400	2000	400	1000	x	400
Candida albicans (ATCC 10231)	1000	600	2000	2000	500	x	1000	1000	600	2000	2000	500	x	1000
Fusarium														

Key: WE = water extract; AC = acetone extract; HX = Hexane extract; DCM = Dichloromethane extract; ME = methanol extract; Ofx = Gatifloxacin; Nys. =Nystatin; x = not determined

*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 developingcountries and represents a constant concern for the foodindustry (Mastroeni, 2002).The most susceptible organisms to the antimicrobial activity of *Temnocalyxobovatus*were *E. coli*, *Staphylococcus aureus*and *Clostridium perfringens.*Different plant extracts have been reported for their antifungal properties (Dzomba and Muchanyereyi 2012**)**. Proteus mirabilis causeswound infections and urinary tract infections in theelderly and young males often following catheterizationor cystoscopy, and it is a secondary invader of ulcersand pressure sores (Cheesbrough, 2000; Parekh andChanda, 2007). The demonstration of activity againstboth gram-negative and gram-positive bacteria and fungiis 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 laboratory isolates is also anindication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms prevalent in hospital environments.

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

Organisations	Zone of inhibition (mm)					
	E	G	EG	E	N	EN
Pseudomonas Aeruginosa (ACCT 9027)	-	-	8	X	x	x
Bacillus subtulis(ATCC 6633)	-	10	12	x	x	x
Staphylococcus aureus(ATCC 6538)	2	4	6	x	x	x
Micrococcus leutus(ATCC9341)	8	-	10	x	x	x
Escherichia coli(ATCC 14169)	10	12	18			

Key: E = Extracts only; G = Gatifloxacin alone; EG = Extrcat/Gatifloxacin; N = Nastatin alone; EN = Extrcat/Nastatine, x = not determined

Organizations Zone of inhibition (mm)

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* (ATCC 14169), followed by the dichloromethane extracts against *S. aureus* (*ATCC 6538*) (MIC 300 µg/mL, MBC 400 µg/mL) (Table 2). Most of the MICvalues 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 values (Table 2) and higher zones of inhibition (Figure 1) for acetone extractsconnotes higher solubility of Phyto-constituents in the acetone compared to the other solvents used. The lowest MIC was recorded for *Escherichia coli*. Differences in MICvalues of antibacterial activity may be attributed to differential susceptibility of bacterial cell walls, which is a result of slight differences inherent in the cell wall structure (Zhao *et al.*,2001). Gram-positive and gram-negative bacteria differ in many features other than the structure of their cell walls, for

example the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria form a barrier to hydrophobic compounds (Mazutti*et al*.,2008). Many similar studies reported differences in antibacterial and antifungal activity of different medicinal plant extracts and the differences were rationalized as due to difference in morphological structure of the cell membranes (Dzomba and Muchanyereyi 2012),.

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 gatifloxacin (30 µg/mL) andnystatin (30 µg/mL) in the presence of the extracts (30 µg/mL). At 30 µg/mL, both gatifloxacinand the extracts had no effect on *P. aerugenosa*, but when combined, there was a remarkable activity (8 mm zone diameter of inhibition). At 30 µg/mL the activity of the extracts and gatifloxacinagainst *E. coli* (*ATCC 14169*) 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-Nystatin combination against the test fungi. At 30 µg/mL, the activity of the extracts alone against *A. niger* (*ATCC 16404*) was 4 mm (zone diameter of inhibition) and that of Nystatin was 14 mm (zone diameter of inhibition), but this activity increased to 16 mm when the extracts and nystatinwere combined. The synergistic effect of some phyto-constituents on antibiotics against some resistant isolates had earlier been reported (Nascimento*et al.,* 2000).

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