Antibiotic Properties of Leaf Extracts of *Senna alexandrina* (L)

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**Abstract:** The antibiotic properties of *Senna alexandrina* (L.) leaves extracts were studied against five bacteria, two molds and yeast by 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 presentation included Saponins, Tannins, Alkaloids and Flavonoids. *Senna alexandrina* (L.) can be used to source antibiotic substances.


**Key words:** *Senna alexandrina* (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 Sharma 2010). 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 (Owolabiet 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 (NPGR, 2008). The most important species are *tinnevelly senna* (*Senna alexandrina* 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. alexandrina* 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 (Gidadoet 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 aeruginosa (ATCC 9027) and three gram-positive bacteria; Micrococcus luteus (ATCC9341), Staphylococcus aureus (ATCC 6538), and Bacillus subtilis (ATCC 6633), and two fungi, Aspergillus niger (ATCC 16404), and Fusarium oxysporum (ATCC 48112) and one yeast Candida albicans (ATCC 10231), (standard laboratory isolates), all obtained from the NAMRO 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 controls for bacteria, nystatin (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 h (for bacteria) and 72 h (for 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 ethanolic extracts. The slightly modified method of (Okerulu and Ani 2001) 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 Senna alexandrina.

<table>
<thead>
<tr>
<th>Extract</th>
<th>pH</th>
<th>% extraction</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Balsams</th>
<th>Anthraquinones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>5.4</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetone</td>
<td>5.2</td>
<td>43</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>5.1</td>
<td>27</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hexan</td>
<td>5.6</td>
<td>48</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>5.3</td>
<td>36</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 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 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 plant has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in plants supports 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 astalet 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 (8 mm 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.
Organisations 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 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 values (Table 2) and higher zones of inhibition (Figure 1) for acetone extracts connotes higher solubility of Phyto-constituents in the acetone compared to the other solvents used. The lowest MIC was recorded for Escherichia coli. Differences in MIC values 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, outer membrane structure and lipopolysaccharides (Miller, 1996). Differences in MIC values 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, outer membrane structure and lipopolysaccharides (Miller, 1996).

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (g/ml) WE</th>
<th>AC</th>
<th>HX</th>
<th>DCM</th>
<th>ME</th>
<th>Gfx</th>
<th>Nys.</th>
<th>MMC (g/ml) WE</th>
<th>AC</th>
<th>HX</th>
<th>DCM</th>
<th>ME</th>
<th>Gfx</th>
<th>Nys.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis (ATCC 6633)</td>
<td>2000</td>
<td>1500</td>
<td>2000</td>
<td>300</td>
<td>1500</td>
<td>200</td>
<td>x</td>
<td>2000</td>
<td>1500</td>
<td>2000</td>
<td>400</td>
<td>1500</td>
<td>300</td>
<td>x</td>
</tr>
<tr>
<td>Staphylococcus aureus (ATCC 6538)</td>
<td>2000</td>
<td>1500</td>
<td>1000</td>
<td>2000</td>
<td>2000</td>
<td>1000 x</td>
<td></td>
<td>2000</td>
<td>1500</td>
<td>1500</td>
<td>2000</td>
<td>2000</td>
<td>1000</td>
<td>x</td>
</tr>
<tr>
<td>Micrococcus leutus (ATCC9341)</td>
<td>2000</td>
<td>600</td>
<td>800</td>
<td>1000</td>
<td>400</td>
<td>1000</td>
<td>x</td>
<td>2000</td>
<td>800</td>
<td>1000</td>
<td>1000</td>
<td>400</td>
<td>1000</td>
<td>x</td>
</tr>
<tr>
<td>Escherichia coli (ATCC 14169)</td>
<td>800</td>
<td>200</td>
<td>1000</td>
<td>1000</td>
<td>400</td>
<td>x</td>
<td>80</td>
<td>x</td>
<td>800</td>
<td>300</td>
<td>1000</td>
<td>2000</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>Candida albicans (ATCC 10231)</td>
<td>1000</td>
<td>600</td>
<td>2000</td>
<td>2000</td>
<td>500</td>
<td>x</td>
<td>1000</td>
<td>x</td>
<td>1000</td>
<td>600</td>
<td>2000</td>
<td>2000</td>
<td>500</td>
<td>x</td>
</tr>
</tbody>
</table>

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 developing countries and represents a constant concern for the food industry (Mastroeni, 2002). The most susceptible organisms to the antimicrobial activity of Temnocalyxobovatuswere E. coli, Staphylococcus aureus and Clostridium perfringens. Different plant extracts have been reported for their antifungal properties (Dzomba and Muchanyereyi, 2012). 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 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.

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

<table>
<thead>
<tr>
<th>Organisations</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas Aeruginosa (ACCT 9027)</td>
<td>E 8 G 4 EG X N x EN</td>
</tr>
<tr>
<td>Bacillus subtilis (ATCC 6633)</td>
<td>E 12 G 10 EG x N x EN</td>
</tr>
<tr>
<td>Staphylococcus aureus (ATCC 6538)</td>
<td>E 6 G 4 EG x N x EN</td>
</tr>
<tr>
<td>Micrococcus leutus (ATCC9341)</td>
<td>E 10 G 8 EG x N x EN</td>
</tr>
</tbody>
</table>

Key: E = Extracts only; G = Gatifloxacin alone; EG = Extract/Gatifloxacin; N = Nastatin alone; EN = Extract/Nastatine; x = not determined
example the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria form a barrier to hydrophobic compounds (Mazzutiet 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) and nystatin (30 μg/mL) in the presence of the extracts (30 μg/mL). At 30 μg/mL, both gatifloxacin and the extracts had no effect on P. aeruginosa, but when combined, there was a remarkable activity (8 mm zone diameter of inhibition). At 30 μg/mL the activity of the extracts and gatifloxacin against 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 nystatin were combined. The synergistic effect of some phyto-constituents on antibiotics against some resistant isolates had earlier been reported (Nascimento et al., 2000).

References