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## Preliminary Investigation on the Phytochemistry and Antimicrobial Activity of *Senna alata* L. Leaves

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**Abstract:** The phytochemistry and antimicrobial effects of water, methanol, chloroform and petroleum ether extracts of *Senna alata* leaves were studied. Extracts tested at a final concentration of 500 µg mL produced *in vitro* antimicrobial activities in assays against clinical isolates of *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonans aeruginosa* and *Bacillus subtilis*. The zones of inhibitions produced by the extracts in agar diffusion assay against the test micro organisms ranged from 8 to 20 mm while the gentamycin antibiotic control produced zones that measured 5 mm. Preliminary phytochemical analysis of the plant extracts showed the presence of phenols, tannins, anthraquinones, saponins, flavonoids.

**Key words:** Phytochemistry, antimicrobial, *Senna alata*, leaves

### INTRODUCTION

Infectious diseases, account for approximately 66.67% of all deaths in tropical countries. In industrialized countries, despite the progress made in the understanding of microbiology and their control, incidents of epidemic due to drug resistant microorganisms and the emergence of hitherto unknown disease causing microbes, pose enormous public health concern. Death from infectious disease in the world ranking 5th in 1981 has become 3rd leading cause of death in 1992, an increase of 58% (Pinner *et al.*, 1989). These negative health trends call for a renewed interest in infectious diseases in the medical and public health communities and renewed strategies on treatment and prevention with the development of new antimicrobials (Iwu *et al.*, 1999).

*Senna alata* (Ring worm cassia or candle bush) belongs to the family Caesalpinaceae, 6-12 feet tall shrub with erect waxy yellow spikes that resembles fat candles before the individual blossoms open, the buds are rounded with 5 overlapping sepals and 5 free or less equal petals narrowed at the base. Leaflets are 8-20, four pairs with lanceolate shape and smooth margin. The fruit is a winged pod and the seeds are small and square. The plant is usually found in secondary vegetation or along riverbanks (Ibrahim and Osman, 1995).

*Senna alata* has been identified as a medicinal plant used in the cure of many ailments and diseases in many parts of the world. The leaves are taken orally as an effective laxative (Ogunti and Elujobi, 1993), while other

researchers claim that an infusion of any part of the plant is laxative/purgative and are used in cases of constipation (Perry and Metzger, 1980). The sap of the leaves is a well-known remedy for ringworm, scabies, ulcers, swelling or inflammation conditions and skin parasites (Crockett *et al.*, 1992). Decoctions of the leaves, flowers, bark and wood of the plant is reported to be effective in the treatment of skin diseases such as purities, eczema and allergy (Abatan, 1990; Benjamin and Lamikaria, 1981). A decoction of leaves and flowers is also used as an expectorant in bronchitis and dyspnoea, as an astringent and also as a mouthwash in stomatitis. In Nigeria and Sierra Leone (West Africa), it possesses a reputation in folklore for laxative properties (Ogunti and Elujobi, 1993; Macfoy and Sanna, 1983). Moreover, the leaves of *S. alata* are used in the treatment of ringworm and other skin diseases in many parts of Nigeria and the use of the plant is widespread throughout Papua New Guinea (Holdsworth, 1992). Other preliminary study on the therapeutic uses of *S. alata* are those by Sansores *et al.* (2000) and Somchit *et al.* (2003).

The objective of this study is to investigate the phytochemistry and antimicrobial activity of *S. alata* leaf extract against the following organisms -*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and high resistant standard strain *Escherichia coli* (J62K<sub>12</sub>). The extracts are traditionally used in Nigeria to treat dermatophytes, bacterial and fungal infections.

## MATERIALS AND METHODS

The plant *Senna alata* was collected in the month of June and August, 2005 from the environment of the University of Benin (Ugbowo Campus) and was identified using herbarium in the Department of Botany, University of Benin.

The test organisms, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and high resistant standard strain *Escherichia coli* (J62K<sub>12</sub>), were obtained from the Department of Pharmaceutical Microbiology, University of Benin, Benin City, Nigeria and were kept as stock cultures in nutrient broth for bacteria and Sabouraud's Dextrose Agar slants for fungi at room temperature 23±2°C until required for use.

### Phytochemistry:

**Extraction of plant material:** Fifteen grams of the powdered leaves sample was put into a beaker in a steam bath and allowed to boil for 30 min. The beaker was removed and left to cool and filtered with Whatman No.1 filter paper to obtain water extract used for phytochemical analysis. The qualitative phytochemical analysis was carried out on the extracts to determine the presence or absence of volatile or essential oils, glycosides, alkaloids, saponin, tannin, flavonoid and anthraquinones, following procedures as outlined by Trease and Evans (1996).

**Extraction:** The leaves of the *Senna alata* plant were dried and made into a fine powder using mortar and pestle. The solvents used for crude extraction were, distilled water, chloroform, methanol and petroleum ether. The weight in grams of plant leaves and volume of solvent used in each case are recorded as 120 g of powdered leaves per 1000 mL of extracting solvents (petroleum ether, water, methanol and chloroform).

Each quantity of the powdered leaves was soaked in each of the solvent for 48 h for maximum dissolution, after which it was sieved and the filtrate was evaporated to a paste using a rotary vapor assembly. The pastes were kept in a labeled container as extract stock.

**Each of the extracts was labeled as follows:** Methanol leaves-ML; Chloroform leaves-CL; Petroleum ether leaves-PL and Water leaves-WL.

**Determination of antimicrobial activity:** The organisms used for this study are *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. J62K12

(*Escherichia coli* standard stains) was used as control organism. Two methods were used: The gutter method and the punch hole method (Stokes, 1975). The gutter method was used to determine activity of each of the extracts on the organisms while the Punch Hole Method was used to measure the zone of inhibition.

**Minimum Inhibitory Concentration (MIC):** Twenty agar plates were prepared and four for each of the extracts were flooded with the same organism. Seven holes were punched in each plate and filled with 0.2 mL in volume containing 500 µg mL<sup>-1</sup> of the extract in different dilution.

Double dilution of the extract was carried out. Double strength nutrient broth of 5 mL was pipetted into universal bottles (seven bottles for each of the extracts) and they were labeled N, 2, 4, 8, 16, 32, 64. Using a sterile graduated pipette, 5 mL of the extract was pipetted into the bottle labeled 2, mixed using a fresh pipette and 5 mL was pipetted into the bottle labeled 4, mixed and 5 mL was pipetted into the bottle labeled 6 until the last bottle labeled 64 while 5 mL was pipetted and discarded. In another bottle, broth only was put in it without the extract, this served as control. The plates were incubated at 37°C for 24 h. The order of concentrations in each of the bottles is as follows: N-500, 2-250; 4-125; 8-62.5; 16-31.2; 32-15.2 and 64-7.8 µg mL<sup>-1</sup>.

## RESULTS

**Phytochemical analysis:** The synopsis of the qualitative phytochemical analysis carried out on the plant is shown in Table 1.

**Volatile oil or essential oil test:** The filtrate from the plant parts gave the characteristic blue color for phenolic compounds thus confirming the presence of phenolic compounds. Also in the case of Eugenol compound a white precipitate was formed which indicates the presence of Eugenol.

**Glycoside test:** The filtrates of the plant parts turned the blue color of the Fehling solution A and B to green, which confirm the presence of glycoside.

Table 1: Synopsis of the qualitative phytochemical analysis carried out on the extract of *Senna alata* leaves

Compound	Leaf
Volatile oil	+
Glycoside	+
Alkaloid	+
Saponins	+
Tannins	+
Flavonoid	+
Anthraquinones	+

+ = positive test

**Alkaloid test:** On addition of 10% KOH solution in alcohol, a violet color that fades rapidly was observed which indicates the presence of tropine alkaloid.

**Saponin test:** The filtrate from the plant parts used produced excessive foam when shaken with distilled water and when mixed with dilute sulphuric acid and boiled, 90% ethanol was added, the initial frothing disappeared. This confirms the presence of saponin and in each case the foaming persisted for more than 15 min which showed a high concentration of saponin.

**Tannin test:** The test for tannin gave bluish precipitate, which confirms the presence of tannins in the plant part used. Test for phenazone was also positive.

**Flavonoid test:** The filtrate from the leaf gave a yellow coloration, which confirms the presence of flavonoid.

**Anthraquinones test:** The filtrate from the leaf gave a pink coloration thus confirming the presence of anthraquinones.

**Antimicrobial activity:** The results of the antimicrobial activity test carried out are expressed in Table (2-4). Table 2 showed that ML and CL were active on some of the organisms while, PL and WL were inactive on all the organisms. *Candida albicans* did not exhibit sensitivity to any of the extracts. ML was active on all the organisms except *Candida albicans*.

From Table 3, the inhibition zones measured showed that J62K<sub>12</sub> had the highest measurement of 11 mm for ML and the least measurement of 4 mm was recorded for CL and WL on *Bacillus subtilis* and *Pseudomonas aeruginosa*. The control antibiotic used, Gentamycin, had inhibition zone of 5 mm while distilled water had no zone.

Table 4 showed the minimum inhibition concentration of the extract by doubling the dilution. Some of the extracts were active on some of the organisms

Table 2: Effect of *Senna alata* extracts on the activity of the microorganisms using the Gutter method

Organism	Extract/activity			
	ML	CL	PL	WL
<i>Staphylococcus aureus</i>	+	+	-	-
<i>Escherichia coli</i>	+	-	-	-
<i>Bacillus subtilis</i>	+	-	-	-
<i>Candida albicans</i>	-	-	-	-
<i>Pseudomonas aeruginosa</i>	+	+	-	-
Standard strain of <i>Escherichia coli</i> J62K12	+	-	-	-
<i>Proteus vulgaris</i>	+	-	-	-

+ = active, - = inactive, ML= Methanol leaves extract, CL = Chloroform leaves extract, PL= Petroleum ether leaves extract, WL =Water leaves extract

Table 3: Zone of inhibition (diameter in mm) shown by the effect of *Senna alata* leaves extracts on microorganisms

Organism	ML	CL	PL	WL	Gentamycin	H <sub>2</sub> O
<i>B. subtilis</i>	10	4	-	-	5	-
<i>P.aeruginosa</i>	8	4	-	-	5	-
<i>E. coli</i>	10	8	-	-	5	-
Standard						
<i>E. coli</i> 62K12	11	-	-	-	5	-
<i>S. aureus</i>	5	6	-	-	5	-
<i>P. vulgaris</i>	5	-	-	-	5	-
<i>C. albicans</i>	-	-	-	-	-	-

No zone., ML= Methanol leaves extract, CL = Chloroform leaves extract, PL= Petroleum ether leaves extract, WL = Water leaves extract,

Table 4: The Minimum Inhibition Concentration (MIC) in µg mL<sup>-1</sup> of *Senna alata* leaves extracts on the microorganisms

Organism	ML	CL	PL
<i>B. subtilis</i>	<7.8	62.5	-
<i>P.aeruginosa</i>	125	250	125
<i>E. coli</i>	125	250	-
Standard			
<i>E. coli</i> J62K12	62.5	-	-
<i>S. aureus</i>	125	62.5	-
<i>P. vulgaris</i>	125	-	-
<i>C. albicans</i>	-	-	-

- = no activity, ML = Methanol leaves extract, CL = Chloroform leaves extract, PL = Petroleum ether leaves extract, WL = Water leaves extract

at very low concentrations while other extracts were active on some of the organisms at high concentrations. ML on *Bacillus subtilis* had activity up to 7.8 µg mL<sup>-1</sup> which is the least concentration and CL was up to 62.5 µg mL<sup>-1</sup>. ML on *Escherichia coli* exhibited MIC of 125 and CL of 250 µg mL<sup>-1</sup>. For *Staphylococcus aureus* ML was 125 and CL 62.5 µg mL<sup>-1</sup>. *Proteus vulgaris* and *Pseudomonas aeruginosa* had MIC of 125 µg mL<sup>-1</sup> for ML and J62K12 exhibited 62.6 µg mL<sup>-1</sup> MIC reaction to the dilution.

## DISCUSSION

Many researchers using different parts of the plant or the entire plant have discovered the medicinal property of *Senna alata*. Giron *et al.* (1991) reported the use of *Cassia alata* for treatment of diabetes, malaria, constipation and stomach pains and the plant has also been implicated to have anti-microbial activity against *Aspergillus brevipes*, *Geotrichum*, *Candidum*., *Penicillium* species and *Fusarium oxysporum* (Adebayo *et al.*, 1991). Ibrahim and Osama (1995) reported that the leaf has antimicrobial activity against *Trichophyton mentagrophytes*, *Mycrosporium canis* and *Mycrosporium gypseum* and Vaijayanthimala *et al.* (2000), reported activity of the leaf water extract on *Candida albicans* while Ikenebomeh and Metitiri (1988) reported antimicrobial activity of *Cassia alata* on *Escherichia coli*, *Aspergillus niger*, *Pennicillium expasum* and *Trichophyton tonsurans*.

The results of the present study showed activity by methanol leaf extract on *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris* and J62K<sub>12</sub> (Standard strain *E. Coli*) at concentration of 500 µg mL<sup>-1</sup> which is in line with the observations of Ogunti *et al.* (1991) on *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 5.0 mg mL<sup>-1</sup>. Also Hasan *et al.* (1988) reported activities of methanol extract on *Bacillus subtilis* and *Escherichia coli* at 1.0 mg mL<sup>-1</sup>, however, observed inactivity of the extract on *Pseudomonas aeruginosa* at the same concentration. These imply that *Pseudomonas aeruginosa* might be inhibited at higher concentration of methanol extract. *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* can be inhibited at concentration up to 1.0 mg mL<sup>-1</sup>.

The results of chloroform leaf extract on *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* at 500 µg mL<sup>-1</sup> indicated activity corresponding with the reports of Ogunti *et al.* (1991) and Hasan *et al.* (1988) on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* at 5.0 and 1.0 mg mL<sup>-1</sup> respectively. This shows that the extract at high and low concentration can inhibit the growth of the organisms. Limserimane and Sirinatana (1983) reported inactivity of the chloroform leaf extract on *Candida albicans*, which agreed with the present study.

Water extract of *Senna alata* leaves was inactive on all the organisms used at 500 µg mL<sup>-1</sup>. Avirutant and Pongpan (1983) and Ahmad *et al.* (1998) reported inactivity of the leaf water extract on *Candida albicans*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 20 mg disk and 200 µg mL<sup>-1</sup> concentration respectively, though Crockett *et al.* (1992) and Vaijayanthimala *et al.* (2000) reported activity of the water extract on *Candida albicans* at 20 mg disk and 75 mg mL<sup>-1</sup>, respectively.

Petroleum ether extract of the leaves was inactive on all the organisms used. Hasan *et al.* (1988) had earlier reported inactivity of the same extract on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*, which indicates the inactivity of the extract on the organisms.

The Minimum Inhibitory Concentration (MIC) of the extracts revealed a decline in activity as the concentrations decreased which implies that the extracts are more active at high concentrations than at low concentrations.

The preliminary phytochemistry investigation carried out showed *Senna alata* to contain some secondary metabolites such as saponins, tannins, phenolic compounds, eugenol, glycosides and anthraquinones.

Generally, secondary metabolites present in plants have been reported by Rabe (2000) to be responsible for their therapeutic activity and Sainsbury and Sofowora (1971) also reported that the volatile oil isolated from *Ocimum gratissimum* had antimicrobial and anthelmintic properties. Thus the antimicrobial activity of *Senna alata* can be attributed to the metabolites present.

## CONCLUSIONS

The results obtained from the test carried out indicates that *Senna alata* can help control infection from *Staphylococcus aureus* which is a major pathogen of human infections varying from food poisoning or minor skin infections to severe life threatening infections, such as *Staphylococcal* bacteraemia and disseminated abscesses in all organs and *Escherichia coli* which causes urinary tract infection, diarrhea, sepsis and meningitis. Phytochemical analysis revealed the various metabolites present in the leaves of the plant used, thus, providing knowledge of the metabolites responsible for its therapeutic quality. However, more research has to be carried out so as to know the longevity of the metabolites in the plant and the effect when low or high concentrations of extracts are used.

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