

## Antimicrobial Qualities of Senna Alata

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**Abstract:** The antimicrobial activity of the root and leaf extracts of the *Senna alata* plant against some infectious bacteria (*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* etc) and fungi (*Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* etc) as well as the physicochemical and microbiological quality of the plant was determined using the cup plate agar diffusion method. The freshly collected fresh mature leaves and roots were chopped into pieces and shade-dried at 32-35°C to constant weight for 5 days. 50g each of the plant parts was coarsely powdered using a mortar and pestle and finely powdered using an electric blender. Each of the powdered air-dried plant material was extracted with water, acetone and methanol. All the extracts demonstrated considerable activity against both Gram negative and Gram positive bacteria and some fungi with the organic extracts showing higher activity than the aqueous extracts. *Streptococcus pyogenes* and *S. aureus* were the most susceptible to all the extracts followed by *Salmonella typhi* and *Escherichia coli*. The most susceptible fungi were *Cryptococcus neoformans* and *Candida albicans* while the least susceptible was *Aspergillus flavus*. The minimum inhibitory concentration and minimum bactericidal concentration of the methanol extracts ranged between 3-10 mg/ml and 25-50 mg/ml for bacteria and fungi respectively. Preliminary phytochemical analysis showed that the extracts contained tannins, saponins, glycosides, flavonoids and phenols. The results obtained show the basis for the local usage of *S. alata* Linn as an antimicrobial. Phytochemical result showed ethanol to be a better solvent for the extraction of the bioactive agents in *Senna alata* which include: glycosides, alkaloids, saponins, tannins, flavonoids and volatile oil. Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms pose an enormous threat to public health. The use of medicinal plants for antimicrobial activities needs to be given more attention.

**Key Words:** Antimicrobial qualities, *Senna alata*, phytochemicals, microorganisms

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### I. Introduction

Infectious diseases are the world's leading human and animal killers (Akinyemi, 2000). The situation is further complicated by the rapid development of multi-drug resistance to available antimicrobial agents (Alalor et al., 2012). In recent years, pharmaceutical companies have focused on developing drugs from natural products (Akinyemi, 2000). Plants still remain the most effective and cheapest alternative sources of drugs (Khan et al., 2001). Drug discovery must be a continuing process if effective chemotherapeutic agents against the rapidly increasing drug resistant bacteria and fungi are to be obtained. The local use of natural plants as primary remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Ibrahim and Osman, 1995). Demands of traditional herbal medicines are increasing day by day not only by the developing countries but also by the developed countries throughout the world. The use of herbal medicine predates the introduction of antibiotics and predates social, economic and religious barriers (Dalziel, 1956). So studies on the ethno-botany, ethnophytopathology and ethnomedicinal uses of our wild medicinal plants and investigations regarding enhanced productivity of medicinal plants is one of the frontier areas of modern research. Ethnobotany is the study of the relationship between people and plants (El-Mahmood and Doughari, 2008). This interdisciplinary field includes studying plants as wild foods and as agricultural crops. Many species of *Senna* possess anti-tumours, laxative, emetic, astringent, anti-pyretic and anti-oxidant properties (El-Mahmood and Doughari, 2008). *Senna alata* -commonly known as "dadmari/candlebrush" having very high medicinal values like, antimicrobial property particularly against fungal dermatophytes and traditionally being used in the treatment of skin infections in man (Amon et al., 2010). Leaf extract is also credited for the treatment of constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes (Daniels et al., 2007). Leaf extract is a good antioxidant and the compound obtained from has been identified as a flavonol compound and named as 'Kaempferol' (Reezal et al., 2002). *Senna alata* is categorized under the family Fabaceae, a pan tropical ornamental shrub, 2-3m high, widely distributed in tropical countries, stretching from Tropical America to India, Fiji, Indonesia, Malaysia and Africa. *Senna alata* Linn (Fabaceae) is an ornamental shrub, which grows well in forest areas of West Africa. It is locally used in Nigeria in the treatment of several infections which include ringworm, parasitic skin diseases (Palanichamy and Nagarajan, 1990). The leaves are reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains, oedema and is also used as a purgative

(Daniels and Knie, 2007 ). Other scientific names of *Cassia alata* are *Senna alata*, *Herpetic alata* and *Cassia bracteata* (Abubakar et al., 2008). *Cassia alata*, is a herb commonly used in Nigeria for the treatment of ringworm, eczema etc. Some of the local Nigerian names are *Ilesko* and *Rinji* in Yoruba and Hausa respectively (Ibrahim and Osman, 1995). Traditionally, the leaves are pounded and rubbed on the skin to cure skin diseases (Pieme et al., 2006). Many reports have shown that some *Cassia* species contain antimicrobial substances, particularly *Cassia alata* (Villasenor et al., 2002). Recent studies revealed that *Cassia alata* has been proven to be effective against bacteria and fungi species (Alalor et al. 2012). They observed that the minimal inhibitory concentration (MIC) values of methanolic extracts of the leaves of *Cassia alata* against *Staphylococcus aureus* and *Bacillus subtilis* were 10mg/ml and 2.5 mg/ml respectively. Idu et al., 2007 observed that preliminary phytochemical analysis of *Cassia alata* showed the presence of phenol, tannins, anthraquinones, saponins and flavonoids. Odunbaku and Lusanya, 2011 also corroborated this study; they further stated that, the plant also had alkaloids and cardenolides. The leaves of *Cassia alata* have been qualitatively analyzed for the presence of anthraquinones: rhein, aloe-emodin, chrysophanol, emodin, and physcion as well as the flavonoid, kaempferol (El-Mahmood and Doughari, 2008). A study in Malaysia (Ibrahim and Osman, 1995) reported that ethanolic extract of the *Senna* plant showed high activity against dermatophytic fungi: *Trichophyton mentagrophytes* var *interdigitale*, *T. Mentagrophytes* var. *mentagrophytes*, *T. rubrum* and *Microsporium gypseum* (MIC: 125mg/ml) and *Microsporium canis* (MIC: 25mg/ml). There are scanty reports on the activity of *Cassia alata* in the form of herbal ointment preparation to the best of the authors' knowledge. The antiseptic property of *Cassia alata*-based herbal soap against common pathogens of the skin has been studied (Esimone et al., 2008). It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates (Ref). (Pieme et al., 2006) have investigated the antifungal and antibacterial activity of *Cassia alata* and have got positive results. This study is aimed at investigating the antimicrobial activity of the root and leaf extracts of the *Senna alata* plant against some infectious bacteria and fungi as well as determining the physicochemical and microbiological quality of the plant.

## II. Materials And Methods

**Collection and identification of plant materials:** The plant *Senna alata* was collected in the morning from the environment of the University of Benin, Ugbowo, Benin City and were identified and authenticated by the Department of Plant Biology and Biotechnology (Herbarium section), University of Benin, Benin City, Nigeria.

### Preparation of Crude and Organic Extracts

The preparation was carried out to simulate the common traditional practice where herbalists often carry out percolation with the application of heat in order to obtain decoctions containing active principles for the desired treatment. Organic solvents (acetone and methanol) were also used for possible enhancement of the extraction process. The freshly collected fresh mature leaves and roots were chopped into pieces and shade dried at 32-35°C to constant weight for 5 days. Subsequently, 50 g of each of the plant parts was coarsely powdered using a mortar and pestle and finely powdered using an electric blender. The powder was transferred into closed containers. Each of the powdered air-dried plant material was extracted with hot water, acetone and methanol. For this purpose, 25 g of each powdered sample was mixed with 100 ml of de-ionized distilled water or organic solvent in a conical flask, plugged, shaken at 120 rpm for 30 min and extracted by warming at 60°C in a shaker water bath at 50 rpm for 2h. This was allowed to cool, and then filtered rapidly through four layers of gauze. The debris was extracted one more time with 100 ml of solvent at 60°C in the shaker water bath at 50 rpm for 2 h and filtered again. The two filtrates were then pooled and a more refined filtration was carried out using Whatman no. 1 filter paper. The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness for aqueous filtrates. The yield of powder was 48 % (aqueous extracts), 32 % (acetone extracts) and 20 % (methanol extracts) for the stem bark and 46 % (aqueous extracts), 30 % (acetone extracts) and 24 % (methanol extracts) for the leaves.

### Phytochemical Analysis

The freshly prepared extracts were subjected to standard phytochemical analysis for different constituents such as tannins, alkaloids, flavonoids, glycosides, saponins and phenols as described by Palanichamy and Nagarajan, 1990.

**Test for Glycosides:** To 1ml of the extract was added 2ml of acetic acid and then cooled in an ice bath at 4°C. To this mixture 1ml of concentrated tetraoxosulphate (vi) acid (H<sub>2</sub>SO<sub>4</sub>) was added dropwise. The formation of an oil layer on top of solution indicated the presence of glycosides (Odebiyi and Sofowora, 1978).

**Test for Alkaloids:** To 3ml of the extract was added 1ml of 1% HCL. This resulting mixture was then treated with few drops of Meyer's reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloids (Ogunkwe et al., 2004).

**Test for Saponins:** Five drops of olive oil was added to 2ml of the plant extract and the mixture shaken vigorously. The formation of a stable emulsion indicated the presence of saponins (Trease and Evans, 1996)

**Test for Tannins:** Two drops of 5% FeCl<sub>3</sub> was added to 1ml of the plant extract. The appearance of a dirty-green precipitate indicated the presence of tannins (Trease and Evans, 1996)

**Test for Flavonoids:** To 1ml of the extract was added 3 drops of ammonia solution (NH<sub>3</sub><sup>+</sup>) followed by 0.5ml of concentrated HCl. The resultant pale brown colouration of the entire mixture indicated the presence of flavonoids (Odebiyi and Sofowora, 1978).

**Test for Steroids:** To 1ml of the plant extract was added 1ml of concentrated tetraoxosulphate (vi) acid (H<sub>2</sub>SO<sub>4</sub>). A red colouration confirmed the presence of steroids (Trease and Evans, 1996).

**Test for Resins:** To 5ml of the extract was added 5ml of copper acetate solution. The mixture was shaken vigorously and allowed to separate. The appearance of a reddish-brown precipitate indicated the presence of resins (Elmahmood and Doughari, 2008).

### Test organisms

Bacterial isolates of *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella flexneri*, *Staphylococcus aureus* and *Streptococcus pyogenes* and the fungal isolates of *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Cryptococcus neoformans* used for this work were sourced from the Department of Medical Microbiology, University of Benin, Teaching Hospital (UBTH), Benin City, Nigeria. All the pure cultures were suspended in nutrient broth and incubated at 37°C for 18 h. Mueller Hinton agar (MHA) and Nutrient agar were used in the test for antibacterial activity while potato dextrose agar (PDA) was used in the test for antifungal activity.

### Determination of antimicrobial activity

Antimicrobial activity of the aqueous and organic extracts of the plant sample was evaluated by the cup plate agar diffusion method (Ibrahim and Osman, 1995). Bacterial cultures were adjusted to 0.5 McFarland's turbidity standard and inoculated onto MHA plates (diameter 15 cm). Prior to the determination of antimycotic activity, all the fungal isolates were first adjusted to a concentration of 10<sup>6</sup> spores/ml. Cultures of *Candida albicans* were suspended in sterile solution of 0.9 % normal saline and the spores of the other filamentous fungi were suspended in Tanquay buffer and then inoculated onto PDA plates. A sterile cork borer was used to make wells of 6 mm diameter on the MHA and of 13 mm diameter on PDA. The width of the zones of inhibition was recorded after incubation. In each of the wells in the culture plates previously seeded with the test organisms, 100µl aliquots of extract dilutions reconstituted in minimum amount of solvent at concentrations of 50 and 100 mg/ml were applied. Sterile glycerol was used as a negative control. Wells containing 20µl aliquots of ciprofloxacin (10µg/ml), streptomycin (30µg/ml), nystatin (10mg/ml) and amphotericin B (10mg/ml) served as positive controls. Bacterial and *Candida albicans* cultures were incubated at 37°C for 18 to 24 h while the filamentous fungal cultures were incubated at 30°C for 36 to 48 h. After incubation, antimicrobial activity was determined by measurement of the width of the zones of inhibition. For all the extracts, the tests were carried out in triplicate against each organism.

### Determination of minimum inhibitory concentration & minimum microbicidal concentration

The minimum inhibitory concentration (MIC) of the methanol extracts was determined for each of the test organisms in triplicate at concentrations of 3, 6, 9, 12, 15, 20, 25, 50 and 100 mg/ml. To obtain these concentrations, 1 ml aliquots of the extracts at double strength concentrations (6, 12, 18, 24, 30, 40, 50 and 200 mg/ml) were added to test tubes containing 1ml of nutrient broth for the bacteria and potato dextrose broth for the fungi. A loopful of the test organisms previously diluted to 0.5 McFarland turbidity standards for bacterial isolates and 10<sup>6</sup> spores/ml for fungal isolates was introduced to the respective tubes. The procedure was repeated using the standard antimicrobials ciprofloxacin, streptomycin, nystatin and amphotericin B instead of the extracts. A tube containing nutrient broth only was seeded with the test organisms to serve as a negative control. Tubes containing bacterial cultures were then incubated at 37°C for 24 h while tubes containing fungal spore cultures were incubated at 30°C for 36h. After incubation the tubes were examined for microbial growth by observing for turbidity. To determine the minimum microbicidal concentration (MMC), which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), a loopful of broth was collected from those tubes which did not show any growth in the MIC determination and inoculated on sterile nutrient agar (NA) for bacteria and Sabourauds Dextrose agar (SDA) for fungi by streaking. To serve as a control, NA and SDA only were streaked with the respective test organisms. Plates inoculated with bacteria were then



<i>Aspergillus flavus</i>	50	50	50	50	NA	NA	25	50
<i>Aspergillus niger</i>	50	50	50	50	NA	NA	25	50
<i>Candida albicans</i>	25	25	35	25	NA	NA	10	50
<i>Cryptococcus neoformans</i>	6	6	13	13	NA	NA	6	13

CIP = ciprofloxacin, STN = streptomycin, AMP B = amphotericin B and NYS = Nystatin.

#### IV. Discussion

Phytochemical analyses revealed that *Senna alata* contained saponins, alkaloids, flavonoids, tannins, phenols and glycosides as shown in Table 1. The antimicrobial susceptibility testing shows that all the extracts (water, ethanol and alcohol) demonstrated significant activity against the bacteria and fungi (Table 2). *Streptococcus pyogenes* and *Staphylococcus aureus* were the most susceptible bacteria to all the extracts followed by *Salmonella typhi* and *Escherichia coli*. The most susceptible fungi species were *Cryptococcus neoformans* and *Candida albicans* while the least susceptible was *Aspergillus flavus*. The results of MIC and MMC determination showed that the MIC and MMC of the extracts ranged between 3-10 mg/ml and 15-50 mg/ml for the bacteria and fungi respectively while those for the antibiotics agent ranged between 3-10 mg/ml and 6-50 mg/ml for the bacteria and fungi respectively as shown in table 3. The highest activity of the plant (roots and leaves) was demonstrated by methanol extraction. Secondary metabolites present in plants have been reported by Rabe (2000) to be responsible for their therapeutic activity. Flavonoids and other physiochemical constitute of the plant also have antimicrobial properties and this is in agreement with Singh and Bhat, 2003 which reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants which. Plant essential or volatile oils and their individual components have been used in traditional systems of medicines for a variety of bacterial infections for centuries and this is also in agreement with Rabe, 2000. Furthermore, it has been demonstrated that antibacterial properties of these oils can be attributed to their hydrocarbon and terpene constituents (Amit and Shailendra, 2006). The presence of glycosides and alkaloids in *Musa sapientum* peels may be attributed to their use by traditional medicine practitioners in healthcare systems in the treatment of some bacterial infections such as cough, fever, cold and venereal diseases (Ibrahim and Osman, 1995). The results of this research highlights the fact that the organic solvent (ethanol) extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted more or only through the organic solvent medium. This observation agrees with the report of other investigators of medicinal plants that organic solvents are more suitable for extraction of phytochemicals (Singh and Singh, 2000; Natarajan et al., 2005). Different solvents have different solubility capacities for different phytoconstituents, hence the differences in the activities of the various extracts (Shanmugam et. al 2008). These bioactive compounds have been reported to be used by plants for protection against bacterial, fungal and pesticidal infections and are responsible for antimicrobial activity (Falodun et al., 2006). Pieme et al., 2006 investigated the antifungal and antibacterial activity of *Cassia alata* and got positive results. Low MIC is an indication of high efficacy of the plant extract while high MIC may indicate low efficacy or possible development of resistance by the microorganisms to the antimicrobial (Shanmugam et al 2008).

#### V. Conclusion

Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms pose an enormous threat to public health. The use of medicinal plants with antimicrobial activities needs to be given more attention to arrest the situation. The demonstration of the antimicrobial activity of *Senna alata* in this work provides scientific basis for its use as a local health remedy. The plant can therefore be used in the treatment of gastrointestinal, urinary tract and wound infections as well as some mycotic infections. There is need for more research on the activity of the extracts against a wider range of bacteria and fungi and on the toxicology and further purification of the extracts for isolation of the pure active constituents. We believe that the result of this study will be an encouragement for further study that will lead to the use of the active components of *Senna alata* in drug preparation in the nearest future.

#### References

- [1]. Abubakar, M. N., Ramanathan, R., Senthil, K. T. (2008). In vitro antifungal activities of *Cassia alata* Linn. Flower extract. *Natural Product Radiance*. **7**(1): 6-9.
- [2]. Alalor, C. A., Igwilo, C. I. and Jeroh, E. (2012). Evaluation of the antibacterial properties of aqueous and methanol extracts of *Cassia alata*. *J. Pharm. and Allied Health Sci.* **2**(2): 40-46.
- [3]. Amao, S. Y., Ajani, R.S. and Oladapo, O. (2010). *Cassia alata* alters Liver Structure in Rat. *Afr. J. Biomed. Res.* **13**:231 – 233.
- [4]. Dalziel, J.M. (1956). *The Useful Plants of West Tropical Africa*. Crown Agents, London, p: 612.
- [5]. Daniels, R. and Knie, U. (2007). Galenics of dermal products vehicles, properties and drug release. *J. Dtsch Dermatol Ges.* **5**:367-381.

- [6]. El-Mahmood, A. M. and Doughari, J. H. (2008). Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn. *Afri J Pharm Pharmacol.* **2** (7): 124-129.
- [7]. El-Mahmood, A.M., Doughari, J. H. and Chanji, F. J. (2008). In vitro antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri*. *Sci. Res. Essays* **3**: 102-105.
- [8]. Esimone, C.O., Nworu, C.S., Ekong, U.S. and Okereke, B. (2008). Evaluation of the antiseptic properties of *Cassia alata*-based herbal soap. *Internet J Altern Med.* **6**(1):34-36.
- [9]. Falodun, A., Okunrobo, L.O. and Uzoamaka, N. (2006). Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn. (Euphorbiaceae). *African Journal of Biotechnology* **5**(6):529-531.
- [10]. Ibrahim, D and Osman, H. (1995). Antimicrobial activity of *Cassia alata* from Malaysia. *J. ethnopharmacol.* **45** (3): 151-156.
- [11]. Idu, M., Omonigho, S. E. and Igeleke, C. L. (2007). Preliminary investigation on the Phytochemistry and antimicrobial activity of *Senna alata* L. Flower. *Pak. J. Biol. Sci.* **10**: 806-809.
- [12]. Khan, M.R., Kihara, M. and Omoloso, A.D. (2001). Antimicrobial activity of *Cassia alata*. *Fitoterapia* **72**: 561-564.
- [13]. Ogunkwe, C.E., Oguzie, E., Unaegbu, C.O and Okolue, B.N (2004). Phytochemical screening on the leaves of *Sansevieria trifasciata*. *J. Chem. Soc. Nig.* **29**(1): 26-29.
- [14]. Odunbaku, O. A. and Lusanya, O.A.F. (2011). Synergistic effect of ethanol leaf extract of *Senna alata* and antimicrobial drugs on some pathogenic microbes. *Adv. Environ. Biol.* **5**: 2162-2165.
- [15]. Odebiyi, O.O and Sofowora, E.A. (1978). Phytochemical Screening of Nigerian medicinal plants part II. *Lloydia* **41**(1): 234-235
- [16]. Palanichamy, S. and Nagarajan, S. (1990). Antifungal activity of *Cassia alata* leaf extract. *J. ethnopharmacol.* **29**(3): 337-340.
- [17]. Pieme C. A., Penlap, V. N., Nkegoum, B., Taziebou, C.L., Tekwu, E.M., Etoa, F.X. and Ngongang, J. (2006) Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (Cesalpiniaceae). *African Journal of Biotechnology* **5** (3): 283-289
- [18]. Reezal, I., Somchit, M.N. and Abdul Rahim, M. (2002). In Vitro Antifungal Properties of *Cassia alata* (Gelenggang Besar). *Proceedings of the Regional Symposium on Environment and Natural Resources 10-11th April 2002, Hotel Renaissance Kuala Lumpur, Malaysia.* **1**: 654-659
- [19]. Shanmugam, K., Holmquist, L., Steele, M., Stuchbury, G., Berbaum, K., Schulz, O., Benavente Garc'ia, O.,Castillo, J. Burnell, J., Garcia Rivas, V., Dobson, G. and Munch, G. (2008). Plant-derived polyphenols attenuate lipopolysaccharide-induced nitric oxide and tumour necrosis factor production in murine microglia intoxication in rats. *Journal of Ethnopharmacology* **66**: 355-361.
- [20]. Singh, B and Bhat, T.K (2003) Potential therapeutic applications of some antinutritional plant secondary metabolites. *Journal Agric . Food. Chem* **51**: 5579-5597
- [21]. Singh, I. and V.P, Singh (2000) Antifungal properties of aqueous and organic solution extracts of seed plants against *Aspergillus flavus* and *A.niger*. *Phytomorphol.* **50**: 151-157.
- [22]. Trease, G.E and W.C Evans (1996). *Pharmacognosy*. Macmillan publishers ltd. Pp 213-832.
- [23]. Villasenor, I.M., Canlas, A.P., Pascua, M.P.L., Sabando, M.N. and Soliven, L.A. (2002). Bioactivity studies on studies on *Cassia alata* Linn. leaf extract. *Phytother Res.* **16**: 93-96.