Phytochemical Screening and Antibacterial Activity of the Leaves of *Senna alata* on Selected Bacteria

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Abstract: Powdered leaves of *Senna alata* were extracted with ethanol and water. The extracts were subjected to phytochemical screening using standard procedures. The extracts were tested for antibacterial activity against four (4) clinical bacterial isolates, which include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli* and *Streptococcus pyogenes,* using agar well diffusion method. The results of phytochemical screening indicated the presence of alkaloids, saponins, anthraquinone, flavonoids, tannins, saponins and glycosides in the two extracts. Results of the antibacterial activities revealed that ethanol fraction was active on *Pseudomonas aeruginosa* at the highest concentration of 500 mg/ml with zone diameter of inhibition of 9 mm, while the aqueous extract was active at the highest concentration of 500mg/ml against *Escherichia coli* and *Streptococcus pygogenes* with zone diameter of inhibition of 11 mm and 10 mm respectively. The results provided evidence that leaf of *Senna alata* has the potential that can be harnessed to produce drugs that can be used to treat ailments caused by these pathogens.

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1 .Introduction

Traditional medicinal plants are therapeutic resource used by the population of the African continent specifically for healthcare, which may also serve as starting materials for drugs infectious diseases account for one-half of all deaths in the tropical countries, as a result, people of all continents have long applied poultice and imbibed infusions of indigenous plants dating back to pre-history for health purposes (Cowan, 1999). It comprises of therapeutic practices in existence for hundreds of years before the development of modern scientific medicine and is still in use today without any documented evidence of adverse effect. According to the World Health Organisation (WHO, 2001) "a medicinal plant is any plant which in one or more of its organ contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs". This definition distinguishes these plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant used for preparing medicines (for example leaves, flowers, seeds, roots, barks, stems, etc) (Anon, 2007). Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Aromatic plants have a pleasant characteristic fragrant smell. The fragrance of these plants is carried in the essential oil fraction. Many aromatic plants are spices. Chandarana *et al.,* (2005)Traditional medicines have been the focus for a wider coverage of primary healthcare delivery in Africa and the rest of the world (Elujoba *et al.,* 2005). Medicinal and aromatic plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds (Sofowora, 1993), which have curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants. Tyler (1999) has reported that plants also contain certain other ingredients. Iwu *et al.,* (1999) reported that the primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. The use of medicinal plants in developing countries as a normative basis for the maintenance of good health has been widely observed (UNESCO, 1996). Furthermore, the increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural remedies (UNESCO, 1998). Moreover, herbal remedies have become more popular in the treatment of minor ailment and also on account of the increasing costs of personal health maintenance.In

the antibacterial activity, it was limited to the determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and determination of the zones of inhibition produced by the leaf extract against some selected bacteria. Only the presence or absence of a general group of organic compounds like saponins, glycosides, anthracenes, alkaloids, flavonoids, tannins, anthraquinones were determined

It was also reported by Aliyu (2006) that *Senna alata* is ethnomedicinally used as laxative in a daily dose of 4 to 8g, blood cleaning agent, cure for digestive system and genitourinary disorders, herpes and rhinitis. They are used as a purgative in a decoction in a single dose of 15 to 20g (Lose *et al.*, 2000).

Specifically, the focus was on *E. coli* and *C. albicans* and the effectiveness of the extracts was evaluated relative to those of standard antibacterial agent chloramphenicol and antifungal agent amphotericin B. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the water extract of *Senna alata* against *E. coli* were 1.6mg/ml and 60mg/ml respectively; corresponding data for chloramphenicol were 2 µg/ml and 10 µg/ml. Similarly, the MIC and Minimum Fungicidal Concentration (MFC) for the extract against *C. albicans* were 0.39 mg/ml and 60 mg/ml in contrast to 0.58 µg/ml and 0.98 µg/ml for Amphotericin B. The data suggest that S. *alata* extracts contain agent(s) which have therapeutic potential and might be useful if isolated and developed for the treatment of opportunistic infections of AIDS patients. (Matinez, 2000).

2. Materials and Methods

The fresh leaves of *Senna alata* free from disease were plucked and gathered mainly from Gwagwalada, Municipal Area Council, Abuja. The leaves were identified by a botanist of the Department of Biological Science, University of Abuja. All glass wares such as conical flask, beaker, pipette, tubes, etc were washed thoroughly and rinsed. Glass wares were sterilized in an autoclave at 121^{o}C for 20mins before use. The work was carried out under aseptic condition. The working bench surface was disinfected with cotton wool soaked in 98% ethanol. Fresh leaves of the plant were washed, sun dried, crushed and grounded to coarse powder using a blending machine. 50 grams of the dried powder was weighed out using a weighing balance respectively for aqueous and ethanolic extract. The powdered leaves were percolated with 300mls of distilled aqueous and ethanol respectively for 78 hours (3 days), after which there was decantation and filtration. They were filtered into containers using Whattman's No. 1 powder using water bath at 40^{o}C. Phytochemical screening was carried out on the aqueous and ethanolic extract for the qualitative determination of phytochemical constituent as described by Trease and Evans (1989). 10% of the sample to solvent was diluted and allowed to stand for 1 hour before filtration.

2.1 Preparation of Medium

Nutrient agar medium was used during the course of this work. Its preparation was done according to the manufacturer's instructions. It was sterilized in an autoclave at 121^{o}C for 15 minutes. After which it was allowed to cool, and was poured into sterile plates and allowed to solidify. A stock culture of the bacteria isolate was obtained from the University of Abuja Teaching Hospital. The test organisms used to determine the antibacterial activity of *Senna alata* leaf are *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli and Streptococcus pyogenes.* The bacteria were maintained throughout the duration of the work on nutrient broth at 37^{o}C. The test organisms were prepared by aseptically transferring a loopful of the organism from stock culture into nutrient broth and incubating at 37^{o}C until use.

2.2 Phytochemical Screening

Phytochemical screening was carried out on the aqueous and ethanolic extract for the qualitative determination of phytochemical constituent as described by Trease and Evans (1989). 10% of the sample to solvent was diluted and allowed to stand for 1 hour before filtration. The extracts were collected and used for the following:

(1) Test for Saponin

5mls of dilute extract of aqueous and ethanol is vigorously shaken respectively for 2mins with 10mls of water (distilled) in 2 test tubes. If frothing occurs and on addition of olive oil emulsion is formed, it indicates the presence of saponin.

(2) Test for Saponin Glycosides

To 2.5mls of the extracts, add a mixture of Fehling solution A and B. A bluish green precipitate shows the presence of Saponin glycoside.

(3) Test for Glycoside

METHOD: Add 2.5ml of dilute H_2SO_4 to 5mls of the extract respectively in a test tube and boil for 15mins, cool and neutralize with 10% NaOH. Add 5mls of Fehling solutions A and B. A brick-red precipitation of reducing sugars indicate presence of glycosides.

(4) Test for Anthracenes

METHOD: Extracts are shaken with equal volume of chloroform and allowed to separate. Brick-red precipitate is formed with anthracenes.

(5) Test for Flavonoids

A few drops of 1% NH_3 solution is added to the aqueous and ethanolic extract respectively of the plant sample in test tubes. A yellow coloration is observed if flavonoid compounds are present.

(6) Test for Alkaloids

METHOD: To the extracts, add a mixture of iodine in potassium iodide and shake vigorously. Deep brown precipitate indicates the presence of alkaloids.

(7) Test for Tannins

METHOD: Boil 5g of each powdered sample in 50ml distilled water of 3 mins on a hot plate. Filter the content. To the filtrate add drops of 10% ferric chloride solution. A blue or green colour indicates the presence of tannins. Trease and Evans (1989)

(8) Test for Anthraquinone

METHOD: To 1g of the powdered plant material, add chloroform and shake it for 5 mins. Filter the content. To the filtrate add 5ml ammonia solution and shake properly. A bright pink colour in the upper aqueous layer indicates the presence of anthraquinone.

2.2 ANTIBACTERIAL ACTIVITY

2.2.1Bioassay Studies

The agar well diffusion method was used for the bioassay. The surfaces of nutrient agar media, prepared according to the manufacturer's instructions were seeded with pure cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli and Streptococcus pyogenes* respectively on separate agar plates and adjusted to 0.5 Macfarland standard (Cheesebrough, 2002). Exactly two drops (0.2ml) each from different concentrations of the aqueous and ethanolic extract that was prepared was introduced into the wells of 6mm diameter which were made on the sensitivity agar using sterile cork borer. Standard antibiotics, Chloramphenicol and Gentamycin which served as positive and negative control were also introduced into the well.

The plates were allowed to stand for 15mins for the extracts to diffuse into the agar after which the plates were inverted and incubated aerobically at 35^{o}C for 24 hours. This was followed by measurement of zone of inhibition by the test organisms around each of the wells and the antibiotic.

2.2.3DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

Determination of the minimum inhibitory concentration (MIC) of the extracts was carried out using the tube diffusion method. Minimum Inhibitory Concentrations of the extract was prepared by serial doubling dilution using injection water to obtain concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 15.625mg/ml, 7.81mg/ml, and 3.91mg/ml. (Lin *et al.*, 1999).16 sterilized test tubes were set up for the different organisms cultured. 2 ml of nutrient broth containing the test organisms was each dispensed into the sterilized test tubes. A quantity (0.1ml) of extract of different concentrations was dropped into the test tubes containing the test organisms.The tubes were tightly corked with foil and were incubated aerobically at 37^{o}C for 24 hours. The tubes were observed after 24 hours of incubation to determine the minimum inhibitory concentration, which is the lowest concentration that showed no evidence of growth.

2.2.4DETERMINATION OF MINIMUM BACTERIAL CONCENTRATION (MBC)

Sterile nutrient agar plates were inoculated with sample from each of the test tubes that showed no evidence of growth. The plates were further incubated for 24 hours at 37^{o}C and observed. The lowest concentration that killed 100% of the inoculum bacteria (no growth on plate) was recorded as Minimum Bactericidal Concentration.

3. Results

Table 1: Results of Phytochemical analysis of extracts from leaves of *Senna alata*

Phytochemical compounds	Ethanolic extract leaves	Aqueous extract leaves
Saponin	+	+
Saponin glycosides	+	+
Glycoside	-	-
Anthracenes	+	+
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Anthraquinone	+	+

+ = Present

- = Absent

Table 2: Zones of inhibition diameter (Mm) formed by isolates in response to ethanolic extracts of *Senna alata* using Agar well diffusion method

Isolates	500 mg/ml	250 mg/ml	100 mg/ml	50 mg/ml	Control (Gentamycin)
S. aureus	6	4	3	2	17
P. aeruginosa	9	5	3	5	14
S. Pyogenes	6	4	4	3	21
E. coli	7	5	4	3	17

Table 3: Zones of inhibition diameter (Mm) formed by isolates in response to aqueous extracts of *Senna Alata* using Agar well diffusion method

Isolates	500 mg/ml	250 mg/ml	100 mg/ml	50 mg/ml	Control (Chloramphenicol)
S. aureus	6	5	0	3	19
P. aeruginosa	4	7	4	2	15
S. pyogenes	10	10	3	0	12
E. coli	11	8	6	0	15

Table 4: Determination of Minimum Inhibitory Concentration (MIC) of aqueous extract

Micro-organism	500	250	125	62.5	31.3
S. aureus	+	+	+ MIC	-	-
P. aeruginosa	+	MIC +	-	-	-
S. pyogenes	+	+	+ MIC	-	-
E. coli	+	+ MIC	-	-	-

+ = Clear
- = Turbid
Turbidity indicates growth. The lowest concentration showing growth is the MIC of the antimicrobial agent for the organism.

TABLE 5:Determination of Minimum Bacterial Concentration (MBC) of aqueous extract.

Micro-organism	500	250	125	62.5	31.3
S. aureus	-	- MBC	+	+	+
P. aeruginosa	-	- MBC	+	+	+
S. pyogenes	- MBC -	+	+	+	+
E. coli		- MBC	+	+	+

+ = Bacteria growth
- = No bacteria growth
This is the lowest concentration that killed 100% of the inoculums bacteria.

4. Discussion

Table 1 shows the result of phytochemical screening carried out on the leaf of *Senna alata* leaf extracts. Phytochemical compounds present were found to be tannins in all the extracts, saponins, alkaloids, anthraquinone, etc. while glycosides were not detected in all the extracts. The antibacterial activity of *S. alata* leaves could be due to the phytochemicals present which have been reported by Dweck (1994) to act as plant protectants against pathogens in the wild. The protection is equally conferred on humans when plant parts are drunk as concoctions, decoctions in ethnomedicine.

Table 2 and 3 shows the antimicrobial activity of both the ethanolic and aqueous extract of *Senna alata* leaves on *P. aeruginosa*, *S. aureus, E. coli,* and *S. pyogenes.* The zone diameter of inhibitions for aqueous and ethanolic extract respectively reveals a dose-dependent antimicrobial activity. The fact that the pathogenic strain of *S. aureus* can cause localized abscesses and septicaemia in humans implies that the ethanolic extract of *Senna alata* could be employed for the treatment of such infections. Alkaloids are compound documented to possess medicinal properties and health promoting effect. (Marjorie, 1999)

Flavonoids are group of phytochemicals found in varying amounts in food and medicinal plants which have been showed to exert potent anti-oxidant activity.

Plants and plant products have been used extensively throughout history to treat medicinal problem. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal

remedies has risen in the developed countries in the last decades. In this connection, plants continue to be a rich source of therapeutic agents. The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industry was possible, because of the large number of phytochemical and biological studies all over the world. Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. Therefore, it is of great interest to have carried out a screening of this plant *Senna alata* in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents.

The activity against the different isolates by leaf extract of *Senna alata* is a justification and explains the basis of its use in traditional medicine. The antibacterial activities of the aqueous and ethanolic extract of the plant against some of the organisms, suggest the presence of bioactive compounds that can serve as antimicrobial agent(s) or lead compound for the synthesis of effective antibacterial agent(s). The potency of *S. alata* against bacteria from these indices gives an evidence for the medicinal value. This study therefore provide basis for the use of this plant as remedy for skin disease, blood cleaning agent, fever, stomach problems, etc. Though *Senna alata* has been described as a plant of low economic value, it is not worthless. Its use in traditional medicine attests to this.

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