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

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Abstract: Preliminary studies on the phytochemistry and extracts of water, methanol, chloroform and petroleum ether, of *Senna alata* flowers were examined for antimicrobial properties. Extracts tested at a final concentration of 500 μg mL^{-1} produced *in vitro* antimicrobial activities in assays against clinical isolates of *Staphylococus aureus*, *Candida albicans*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonans aureginosa* and *Bacillus subtilis*. The zones of inhibitions produced by the extracts in agar diffusion assay against the test micro organisms ranged from 4 to 10 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, anthraquinoes, saponins, flavonoids.

Key words: Phytochemistry, antimicrobial activity, *Senna alata* L., flowers

INTRODUCTION

Historically, plants have provided a good source of anti-infective agents with compounds which are highly effective instruments in the fight against microbial infections. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDs infection. Plants containing protoberines and related alkaloids, picrolema types indole, alkaloids and garcinea biflavones used in traditional African system of medicine have been found to be active against a wide variety of microorganisms (Iwu *et al*., 1999). Though soil microorganisms produce most of the clinically used antibiotics, higher plants have also been a source of antibiotics (Omar *et al*., 2000).

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 five overlaping sepals and five free or less equal petals narrowed at the base. The flowers are bisexual and zygomorphic. The ovary is superior with marginal placentation. The leaves are large, bilaterally symmetrical with even-pinnate. 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 Osma, 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 internally as an effective laxative (Ogunti and Elujoba, 1993). Decoction of the leaves, flowers, bark and wood of the plant are reported to treat skin diseases such as purities eczema and itching (Abatan, 1990; Benjamin and Lamikaria, 1981), an infusion of the flowers is used for asthma and bronchitis (Ibrahim and Osman, 1995). A decoction of flowers is also used as an expectorant in bronchitis and dysporea, 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 (Macfoy and Sanna, 1983; Giron *et al*., 1991; Ogunti and Elujobi, 1993; Coee and Anderson, 1996). A lot of research works with respect to antimicrobial property of *Senna alata* leaves have been conducted by many researchers, but not much work has been done on the flowers. The plant has been reported to have anti-microbial activity against *Aspergillus brevipus*, *Geotrichum candidum*, *Penicillium* species and *Fusarium oxysporum* (Adebayo *et al*., 1991), while Ibrahim and Osama (1995) reported the leaf to have antimicrobial activity against *Trichophytorn mentagrophytes*, *Mycrosporum canis* and *Mycrosporum gypseum*. Vaijayanthimala *et al*. (2000) reported activity of water extract leaf on *Candida albicans* and Ikenebomeh and Metitiri (1988) reported antimicrobial activity of *Cassia alata* on *Escherichia coli*, *Aspergillus niger*, *Pennicillium expasum* and *Trichophyton tonsurans*.

Plants contain chemical compounds that may be in one way or another responsible for their healing properties and other functions. The chemical compounds

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are secondary metabolite of which at least twelve thousand have been isolated (Hasan *et al*., 1988). In many cases, these compounds are responsible for plants flavor e.g. terpenoid capsaicin from chili pepper and some herbs and spices used by human to season food, yield useful medicinal compounds. These useful medicinal phytochemical compounds are phenols, alkaloids, saponins, tannins, glycosides, anthraquinones etc. They have been shown to be present in plants like *Baisses axilasis*, which contains phenolic compounds, eugenol and glycosides (Ohwofahworaye, 1999). Other examples are Warburgia solution, which contains the compound, sesquiterpenoid, which has antimicrobial activity (Rabe, 2000) and *Occium gratissium*, which produces volatile oil, which also has antimicrobal and anthelminthic properties (Sainisbury and Sofowora, 1971).

This study is aimed at determining the phytochemistry and antimicrobal activity of the flower of *Senna alata* against the following organisms-*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Protus vulgaris* and high resistant standard strain *Escherichia coli* ($J62K_{12}$).

MATERIALS AND METHODS

Sample collection: The plant *Senna alata* was collected from the environment of University of Benin, Ugbowo, Benin City. Identification of the plant was by the Herbarium in Department of Botany, University of Benin, Benin City. Nigeria.

Phytochemistry

Extraction of plant material for chemical analysis: Test for volatile or essential oils, glycoside, alkaloids, saponins, tannins, phenazone test, flavonoids and anthraquinones, was according to procedures outlined by Trease and Evans (1996). Fifteen gram of the powered plant 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 before the chemical analysis.

The flowers of the plant for microbiological analysis was 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. One hundred and twenty grams of plant sample were soaked in 1000 mL each of solvent (petroleum ether, hot water, methanol and chloroform). Powdered plant 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 stocks as follows-Methanol Flower-MF; Chloroform Flower-CF; Petroleum ether Flower-PF and Water Flower-WF.

Determination of antimicrobial activity: The organisms used for study were *Bacillus subtilis, Escherichia coli, Candida albicans, Staphylococcus aureus, Pseudomonas aureginosa, Proteus vulgaris* and high resistance standard strain *Escherichia coli* ($J62K_{12}$) that was used as control organism. Two methods were used-The gutter method and the punch hole method. The gutter method was used to determine activity of each of the extracts on the organisms. Each of the organisms was streaked across a Petri dish containing sterilized nutrient agar and was cut open at the middle forming a gutter, which was filled with the plant extracts. The plates were incubated for 24 h at 37°C, after which, they were examined for growth.

Punch hole method: Punch Hole Method (Stokes, 1975) was used so as to measure the zone of inhibition. Twenty Petri dishes were poured with already sterilized nutrient agar to the level of obtaining a standard well and allowed to set. The organisms dissolved in nutrients broth was poured into the set plate and the excesses were poured away in order to ensure uniform distribution of the organism on the Petri dish. A sterile cork borer, 10 mm in diameter was used to punch holes on the agar in the Petri dish. Each of the holes were filled with the extracts and kept in the incubator for 24 h at 37° C. The active extracts had zones of inhibition, which were measured using a meter rule, by measuring two points around the zone and the average taken.

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 volume containing 500 μg mL^{-1} 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 8 until the last bottle labeled 64, 5 mL was then pipetted and discarded. In another bottle, broth only was put, 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^{-1}.

RESULTS

Phytochemical analysis: 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.

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.

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

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

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

The filtrate from the flower gave a yellow coloration, which confirms the presence of flavonoid (Table 1).

The filtrate from flower gave a pink coloration thus confirming the presence of anthraquinones.

Antimicrobial activity: Table 2 showed that MF, CF and WF were active on some of the organisms while PF was inactive on all the organisms. *Candida albicans* did not exhibit sensitivity to any of the extracts.

From Table 3, the inhibition zones measured showed that $J62K_{12}$ and *E. coli* had the highest measurement of 10 mm for MF was on was recorded for *E. coli* and resistance *E. coli* ($J62K_{12}$) and the least measurement of 4 mm for WF and MF was on *Bacillus subtilis* and *Staphylococcus aureus*, respectively. The control antibiotic used; Gentamycin had inhibition zone of 5 mm and water had no zone.

Table 1: Summary of the phytochemical analysis result

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

Table 2: Gutter method showing activity and inactivity of the extracts

Organism	MF	CF	PF	WF
Bacillus subtilis	+	+	-	+
Escherichia coli	+	+	-	-
Standard strain of *Escherichia coli* $J62K_{12}$	+	-	-	-
Proteus vulgaris	+	-	-	-
Pseudomonas aureginosa	+	-	-	-
Staphylococcus aureus	+	-	-	-
Candida albicans	--	-	-	-

+: active, -: inactive, MF = Methanol flower extract., CF = Chloroform flower extract, PF = Petroleum ether flower extract, WF = Water flower extract

Table 3: Punch hole method showing Inhibition zone (diameters mm) produced by flower extracts of *Senna alata*

Organism	MF	CF	PF	WF	Gentamycin	H_2O
B. subtilis	8	-	-	4	5	-
E. coli	10	-	-	-	5	-
Standard *E. coli* $J62K_{12}$	10	-	-	8	5	-
P. vulgaris	5	-	-	-	5	-
P. aureginosa	5	-	-	-	5	-
S. aureus	4	-	-	-	5	-
C. albicans	-	-	-	-	-	-

- No zone., MF = Methanol flower extract., CF = Choroform flower extract., PF = Petroleum ether flower extract., WF = Water flower extract

Table 4: Minimum Inhibition Concentration (MIC) in μg mL^{-1} of *Senna alata* flower extracts on the organisms

Organism	MF	WF
B. subtilis	15.2	125
P. aureginosa	250	-
E. coli	250	-
Standard *E. coli* $J62K_{12}$	62.5	250
S. aureus	125	-
P. vulgaris	125	-
C. albicans	-	-

- Implies No zone., MF = Methanol flower extract.,WF = Water flower extract

Table 4 showed the minimum inhibition concentration (MIC) of the extraction by doubling the dilution. Some of the extracts were active on some of the organisms at very low concentration while others were active on some of the organisms at high concentrations. MF on *Bacillus subtilis* had activity up to 15.2 μg mL^{-1}, which is the least concentration and WF was up to 125 μg mL^{-1}. MF on *Escherichia coli* exhibited MIC of 250 μg mL^{-1}. For *Staphylococcus aureus* and *Proteus vulgaris,* MF was 125 μg mL^{-1} for each of the orgs. and $J62K_{12}$ exhibited 62.5 μg mL^{-1} reaction to the dilution.

DISCUSSION

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 Sainisbury and Sofowora (1971) also reported that the volatile oil isolated from *Ocimum gratisium* have antimicrobial and anthelminthic properties. Petroleum ether flower extract was inactive on all the organisms used while chloroform flower extract and petroleum ether was inactive on all the organisms except *Bacillus subtili* and *Escherichia coli* indicating the impotency of the extracts on the organisms used.

Vaijayanthimala *et al.* (2000) reported activity of leaf water extract on *Candida albicans* but the flower extracts showed inactivity against this organism. Methanol and chloroform flower extract were active against *Bacillus subtilis* and *Escherichia coli*. Ikenebomeh and Metitiri (1988) have also reported on the antimicrobial activity of *Cassia alata* on *E. coli* and fungi. Water extract of *Senna alata* flower showed no antimicrobial activity against the organisms used except *Bacillus subtilis* but other reports showed the antimicrobial activity of leaf water extract against several microorganisms (Adebayo *et al*., 1991; Ibrahim and Osama, 1995). 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 results of the present work showed activity by methanol flower extract on *Bacillus subtilis*, *Pseudomonas aureginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus valgaris* and $J62K_{12}$ (Standard strain *E. coli*) at concentration of 500 μg mL^{-1}. Thus the antimicrobial activity of *Senna alata* flower can be attributed to the metabolites present.

CONCLUSION

The results obtained from the test carried out indicates that *Senna alata* flowers can help control diseases caused by *Staphylococcus aureus* which is a major pathogen for human infections varying from food poisoning or minor skin infections to severe life threatening infections, such as Staphylococcal bacteremia and disseminated abscesses in all organs and *Escherichia coli* with other coli forms which cause urinary tract infection, diarrhea, sepsis and meningitis. Phytochemical analysis revealed the various metabolites present in the flower 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 of low or high concentrations usage.

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