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Phytochemical and Antibacterial Potentials of *Senna tora* Leaf and Seed Extracts against Some Clinically Isolated Bacteria

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Abstract

This study was carried out to determine chemical constituents and antimicrobial activities of extracts obtained from the leaves and seeds of *Senna tora*. The chemical compositions of the leaf and seed extracts were profiled using GC-MS while Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram negative bacteria (*Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*) were used to test for antibacterial potentials. GC-MS analysis showed that the main components in the leaf extract of *S. tora* were cis-oleic acid (29.4%), 1, E-11, Z-13-octadecatriene (13.4%), palmitic acid (13.3%), 1,E-8,Z-10-pentadecatriene (11.4%) and stearic acid (11.0%) while methyl-1-allyl-2-hydroxycyclopentanecarboxylate (20.0%), 6,9- pentadecadien-1-ol (20.0%), cis-oleic acid (16.2%), methyl-7-hexadecenoate (7.5%) and palmitic acid (6.5%) were the most abundant components in the seed extract. The leaves showed a higher inhibitory effect than the seeds, with a zone of inhibition mean value that ranged from 12.3-18.5 mm, while that of seeds ranged from 10-16.5 mm. *Klebsiella pneumoniae* exhibited the highest susceptibility (18.5 mm), while *Salmonella typhi* showed the lowest (10 mm). The results of this study revealed that the leaves and the stems of the plant contained some medicinally active phytochemicals that may be important as antimicrobial agent.

Keywords: *Senna tora*; Extract; Phytochemical; Antimicrobial activities; Pathogenic organisms

Introduction

The search for secondary metabolites from plants as substitutes for synthetic drugs has received much attention. This may be because medicinal plants have been found to have active therapeutic properties against many diseases with less or no side effect compared to synthetic drugs. Recently, treatment of diseases or infections with different medicinal plants has been a predominant practice among the people most especially in the rural areas [1,2]. The rural dwellers relied on herbal medicine, since they cannot afford the orthodox medicine and this practice has been providing the needed cure for their ailments [3,4]. Plant-based systems continue to play an essential role in healthcare, and their use by different cultures has been extensively documented. Phytochemicals have been for age's rich sources for successful drugs discovery, and still represent an important pool for the identification of novel drug. Scientific evaluation of medicinal plants provides evidence-based alternative medicines which form the basis of herbal drug industry and discovery of drug targets in the pharmaceutical industry. The main asset of medicinal plant-based drug discovery is the existence of ethnopharmacological information providing information for compounds therapeutically effective in humans [5-7].

Senna tora also known as *Cassia tora* is a well-known plant in Africa and Asia [8]. *S. tora* has long pinnate leaves; each leaf has three pairs of leaflets that are opposite, ovate, oblong and oblique at the base. The yellow-colored flowers are bearded in the axil of the leaves. The flowers consist of half inch diameter five petals. The seeds of *S. tora* are rhombohedral and brown in colour. The *S. tora* gets flowers in the rainy season and the fruits in the winter. *S. tora* leaves, seeds and roots are utilized as food ingredients and additives [9,10]. It possesses wide range of pharmacological activities. *S. tora* is a medicinal plant known for its laxative, antihepatotoxic, antimutagenic and antiperiodic properties. It is also useful for treatment of leprosy, ringworm, bronchitis, cardiac disorders, ophthalmic diseases, skin diseases, cough, hepatic disorder, liver tonic, haemorrhoids [11]. It was reported that seeds of *S. tora* has antioxidant activity and contain many active substances including chrysophenol, emodin, and rhein [12-14]. Many medicinal properties such as, antihepatotoxic, and antimutagenic activities have been attributed to this plant [11]. In view of the increasingly difficult problems of microbial resistance to most antibiotics, medicinal plants are now being considered as credible alternatives for the treatment of diverse infections [15,16]. Therefore, this study was carried out to determine chemical constituents and antimicrobial activities of extracts obtained from the leaves and seeds of *Senna tora* using methanol, hot water and cold water at different concentrations.

Materials and Methods

Collection and preparation of sample materials

Mature, fresh and healthy samples of *S. tora* were collected from Benja Village, Ota and Pakoto, Ifo, Ogun State. The plant was identified and authenticated as *Senna tora*. Fresh samples (leaves and seeds) of the plant were rinsed with sterile water; air dried for few days and pulverized using a mortar and pestle. The extraction was carried out using 20 g each of the samples in three different solvents (pure methanol, hot water and cold water) in ratio 1:10. The extracts were

then filtered through a Whatman No 1 filter paper. The filtrates were concentrated in an oven at 50°C, then stored in vials and kept in a refrigerator until used.

GC-MS analyses

The extracts of *S. tora* was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m × 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (temperature at 60°C was held for 1.0 min, raised to 180°C for 3 min and then finally to 280°C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250°C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200°C; interface temperature, 250°C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature [17].

Preparation of stock solutions

The amount of dried metabolites that were obtained from the extracts were dissolved in Tween 20 thereby making extracts of different concentration as follows; 512, 256, 128, 64, 32, 16, 8, 4 mg/ml.

Collection and maintenance of organisms

The organisms used for this study were all human pathogenic organisms from clinically isolated bacteria. They were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Salmonella typhi*. The organisms were collected on sterile nutrient agar slants and incubated at 37°C for 24 h. They were then kept as stock cultures in the refrigerator at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmatory purposes.

Preparation of bacteria suspension

Fresh and pure bacteria isolates grown in Nutrient agar were mixed with 10 ml sterile distilled water and the turbidity of the suspension was compared with that of 0.5 McFarland standard equivalents to 1.0×10^8 cfu/ml.

Screening for antibacterial activities

The antibacterial potentials of the leaf and seed extracts of *S. tora* on the clinically isolated bacteria were determined by agar well diffusion method. Nutrient agar plates were inoculated with the various isolates from stock cultures using a sterile swab in order to ensure even distribution of the inoculum. 5 mm equidistant wells were made in the inoculated agar and the wells were filled with each concentration of the extracts. They were then kept in the refrigerator for 1 h for adequate absorption of the extracts into the seeded agar and then incubated at 37°C for 24 h. The diameter of the zone of inhibition millimetre (mm) around each well was measured using transparent ruler.

Antibiotic sensitivity test

This method served as a control in order to compare that of the plant's antimicrobial test. A multi-antibiotic disc bearing eight different antibiotics was used; Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Augmentin (50 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg). Nutrient agar plates were inoculated with the various prepared isolates from stock cultures using a sterile swab in order to ensure even distribution of the inoculum. After 30 min. of applying the disc, the plates were inverted and incubated at 37°C for 24 h. After which clear zones of growth inhibition were measured with the aid of a ruler in millimetres and recorded [17].

Results and Discussion

Chemical composition of the leaf and seed extracts

The GC-MS analyses of the leaf and seed extracts led to the identification of 19 and 23 constituents representing 99.0% and 98.4% of the extract of *S. tora* leaf and seed, respectively. The compounds, retention indices, and percentage compositions were given in Table 1, where the identified components were listed in order of their retention indices. In this study, the leaves and seeds of *S. tora* were investigated for the chemical composition of their crude extracts. The extracts were subjected to GC-MS analyses for their detail identification of components. The main components in the leaf extract of *S. tora* were cis-oleic acid (29.4%), 1,E-11,Z-13-octadecatriene (13.4%), palmitic acid (13.3%), 1,E-8, Z-10-pentadecatriene (11.4%) and stearic acid (11.0%) while methyl-1-allyl-2-hydroxycyclopentanecarboxylate (20.0%), 6,9-pentadecadien-1-ol (20.0%), cis-oleic acid (16.2%), methyl-7-hexadecenoate (7.5%) and palmitic acid (6.5%) were the most abundant components in the seed extract of *S. tora*. The extracts of *S. tora* contained some compounds that are the same, but the leaf extract had higher percentage of cis-oleic acid, 1, E-11, Z-13-octadecatriene, palmitic acid, stearic acid, lauric acid and myristic acid than the seed extract as shown in Table 1, but both parts contained more fatty acid than other classes of organic compounds. The chemical compositions of the leaf and seed extracts of *S. tora* were different from the essential oils components of *S. alata*, *S. occidentalis* and *S. hirsute*. The main components of *S. alata* essential oil were ar-turmerone (13.5%), β-caryophyllene (7.3%), (E)-phytol (7.0%) and 6,10,14-trimethyl-2-pentadecanone (6.8%). (E)-phytol (26.0%), hexadecanoic acid (17.3%), 6, 10, 14-trimethyl-2-pentadecanone (9.9 %) were the quantitatively significant constituents in *S. occidentalis*; while (E)-phytol (30.8%) and pentadecanal (21.7%) were the main components of *S. hirsuta* essential oil [18].

Antibacterial activities

The three extraction methods that is, the methanol, hot water and cold water were used for each plant part and their effects were tested on selected clinical isolates. Table 2 shows the antimicrobial activities of the extracts on the clinical isolates. The findings of this study, showed that leaves had higher inhibitory effect than the seeds, with a zone of inhibition that ranged from 12.3-18.5 mm, while that of seeds ranged from 10.0-16.5 mm. These activities were due to synergic effects of the secondary metabolites in leaves and seeds of this plant. This study also showed that there was an inhibitory effect of the tested plant parts on the organisms.

Compound	Retention Index	Percentage Composition	
		Leaf	Seed
γ-butyrolactone	825	0.7	-
methyl-4-methyloctanoate	1118	-	2.0
1-pyrrolidinylacetic acid	1168	0.8	-
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	0.5	-
α-pipecolinic acid	1283	0.2	-
p-vinylguaiacol	1293	0.3	-
methyl-1-allyl-2-hydroxycyclopentanecarboxylate	1378	-	20.0
undec-10-enoic acid	1461	4.9	-
1,E-8,Z-10-pentadecatriene	1518	11.4	-
lauric acid	1570	1.5	0.7
Z-9-tetradecenal	1609	1.0	-
myristic acid	1769	0.9	0.5
6,9-pentadecadien-1-ol	1771	-	20.0
methyl-14-methylpentadecanoate	1814	2.0	2.6
1,E-11,Z-13-octadecatriene	1817	13.4	5.3
isopropylmyristate	1824	-	0.6
methyl-7-hexadecenoate	1886	-	7.5
2-dodecyl-1,3-propanediol	1934	-	0.5
palmitic acid	1968	13.3	6.5
octyl-10-undecenoate	2067	-	0.1
9,12-octadecadien-1-ol	2069	-	2.0
vinyl stearyl ether	2075	-	0.1
stearic acid, methyl ester	2077	-	1.8
methyl-n-octadecanoate	2077	1.0	-
methyl-11-octadecenoate	2085	3.0	4
methyllinolelaidate	2093	3.0	1.2
n-nonadecanol	2153	-	0.1
stearic acid	2167	11.0	2.7
cis-oleic acid	2175	29.4	16.2
methyl-11,14-icosadienoate	2292	-	1.2
glycerol-1-palmitate	2482	0.7	-
octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	2694	-	2.7
1,1-bis (dodecyloxy)hexadecane	4085	-	0.1
Percentage Total		99	98.4

Table 1: Chemical Composition of the Leaf and Seed Extracts of *Senna tora*.

The plants exhibited antibacterial properties against both Gram positive and Gram negative organisms which agreed with the result obtained by [19]. The methanol extract showed the highest inhibitory effect on the tested clinical isolates and this is in agreement with previous work carried out by [20]. Then, hot water extract showed less inhibitory effect compared to methanolic extract while cold water extract showed the least inhibitory effect. However, there was statistically significant difference in the effects of both the leaves and seeds and this is in accordance with a test carried out by [19] the leaves showed higher antibacterial effects. In addition, the effects observed was dependent on the concentration of the extracts, however, there was no correlation between the antibacterial activities of leaves or seeds at different concentration of the extracts. The effects measured was also dependent on the extraction method (cold water, hot water and methanol) used and the parts of the plant used. Table 3 shows the susceptibility of the test organisms to different antibiotics. The tested bacteria were inhibited by at least one antibiotic. They were all resistant to Augmentin, Ampicillin, Ceftazidime, Cefuroxime and Gentamicin. Moreover, the findings from this study indicated higher resistance pattern exhibited by these organisms to synthetic antibiotics in comparison to the high inhibitory effect of *S. tora* extract against these organisms. Therefore, if the plant can be adequately harnessed and studied, it can be used as a good antibacterial drug against some of these pathogens as discovered in this study. It was observed that the Gram positive bacteria were more susceptible than the Gram negative bacteria. This is in agreement with an earlier study carried out by [21], which reported that plant extracts are more active against Gram positive bacteria than Gram negative bacteria.

Bacteria Isolates	Leaves			Seeds		
	Methanol	Hot H_2O	Cold H_2O	Methanol	Hot H_2O	Cold H_2O
E. faecalis	18.0	16.0	15.3	16.5	11.8	11.8
S. aureus	17.0	16.3	15.8	14.0	14.8	14.5
K. pneumoniae	18.5	17.8	13.5	12.7	13.3	14.8
E. coli	18.0	18.0	12.3	15.3	11.0	11.4
P. aeruginosa	17.5	17.8	14.3	15.3	13.2	11.1
S. typhi	18.0	17.0	14.5	12.8	10.0	15.3

Table 2: Antibacterial Activities of Leaf and Seed Extracts of *Senna tora*.

Bacteria Isolates	AUG (30 µg)	NIT (300 µg)	AMP (10 µg)	CAZ (30 µg)	CRX (30 µg)	GEN (10 µg)	CPR (5 µg)	OFL (5 µg)

E. faecalis	R	S	R	R	R	R	S	S
S. typhi	R	I	R	R	R	R	S	S
K. pneumoniae	R	R	R	R	R	R	S	I
E. coli	R	S	R	R	R	R	S	S
P. aeruginosa	R	R	R	R	R	R	S	S
S. aureus	R	R	R	R	R	R	S	S

Table 3: Antibiotics susceptibility of the tested clinical isolates. AUG: Augmentin; NIT: Nitrofurantoin; AMP: Ampicillin; CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CPR: Ciprofloxacin; OFL: Ofloxacin; S: Susceptible; R: Resistant; I: Intermediate.

Conclusion

This study showed that the plant, *S.tora* exhibited notable inhibitory activities against all the tested pathogenic bacteria. Therefore, plant can be used in developing antibacterial drug in combating multidrug resistant bacteria. The antibacterial activities of this plant could be attributed to the synergic effects of the phytochemicals in the plant. The study also supported the traditional use of the plant for treatment of some diseases. More research needs to be carried out in order to investigate the modes of action, safety and dosage of the plant.

Competing Interests

The authors declare that they have no competing interests.

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