

Full Length Research Paper

Phytochemical screening and antibacterial activity of the leaf and root extracts of *Senna italica*

Y. U. Dabai¹, A. H. Kawo^{2*} and R. M. Aliyu³

¹Department of Public Health and Preventive Medicine, Usmanu Danfodiyo University, PMB 2346, Sokoto, Nigeria.

²Microbiology Unit, Department of Biological Sciences, Bayero University, PMB 3011, Kano, Nigeria.

³Department of Applied Chemistry, Usmanu Danfodiyo University, PMB 2346, Sokoto, Nigeria.

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The leaf and root extracts of *Senna italica* were screened for phytochemical properties and antibacterial activity using standard methods. Alkaloids, steroids and flavonoids were detected in aqueous-methanol, n-hexane and aqueous extracts while tannins, glycosides and saponins were not detected in all the extracts. Sensitivity testing of the extracts showed a strong activity against all the test bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*), which increased with increasing concentration of the extracts (30, 60, 90 and 120 mg/ml). Thus, inhibition zones of 20.0 ± 0.82 , 32.0 ± 0.50 , 32.0 ± 0.50 , 33.0 ± 1.64 and 31.25 ± 0.25 mm were recorded at the highest concentration of 120 mg/ml for the leaf extracts against *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa* and *S. pneumoniae* respectively. Similar trend was observed for the root extracts. Therefore, on the ground of the pronounced activity of the extracts against the test bacteria as well as the presence of alkaloids, flavonoids and steroids in the extracts, it could be suggested that this plant has a potential as a source of therapeutic agents. This supports the traditional use of the plant in curing human diseases. It is therefore suggested that further studies be carried out using different solvents as well as to isolate, purify and identify the active compounds present in the extracts with a view to justifying these claims.

Key words: *Senna italica*, phytochemical screening, antibacterial activity, clinical isolates.

INTRODUCTION

The use of plants as source of remedies for the treatment of diseases dates back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). Human disease management in Nigerian history also provides evidence of the relationship of plants and medicine (Ayandele and Adebisi, 2007). Sofowora (1984) projected the importance of medicinal plants and traditional medicine. However, research and development on medicinal plants have not advanced to the stage of impacting positively on the health system in Nigeria like other African countries

(Odugbemi, 2008). Plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as secondary metabolites. According to Lawal et al. (2005), Magaji and Yaro (2006) as well as Kawo et al. (2009, 2011), phytochemical components are responsible for both pharmacological and toxic activities in plants. These metabolites are said to be useful to the plant itself but can be toxic to animals including man. *Senna italica* ('Filasko' in Hausa) with synonyms *Cassia italica* and *Acacia abovata* belongs to the family of Fabaceae. It is a small herb that grows to about two feet high with smooth and pale green stem having long spreading branches. It has peculiar odor and sweetish taste. Ethno-medically, the decoction of the entire plant or its leaves has been reported to be used as laxative and purgative. The plant also acts as an expectorant against constipation, rheumatic and intestinal disorders (Al-Said, 1993). It is one of the common herbs found in the herbs market of this region. The plant

*Correspondence author. E-mail: ahkawo@yahoo.com. +234-802-3153895.

(*Senna italica*) is widely used to cure stomach ailments as revealed by some herbalists in Sokoto (personal communication) that it is used either alone or along with some substance such as honey or potash. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values (Nastro et al., 2000). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1996; Hammer et al., 1999). Plants containing terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Kubmarawa et al., 2008). The leaves and roots of *S. italica* are used for the treatment of typhoid fever, stomach problem and urinary infection (Al-Said, 1993). In the northern parts of Nigeria especially in Sokoto, the root alone is used trado-medically in the treatment of bacterial diseases. This was what initiated the reason for the present study with a view to justifying these claims.

MATERIALS AND METHODS

Collection and identification of the plant material

The whole plant (*Senna italica*) was obtained from Dundaye village in Wamakko local government area of Sokoto State, north-western Nigeria. The identity of the plant was authenticated by a taxonomist at the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The root obtained from the plant was open air-dried under the shade pulverized into a moderately coarse powder (using a wooden pestle and mortar) and the leaves were dried under the shade to avoid possible damage to phytochemical reaction of the target constituents. They were stored in air-tight containers at room temperature until required for use (Onoruvwe and Olorunfemi, 1998).

Test microorganisms

The test microorganisms used were *P. aeruginosa*, *E. coli*, *S. aureus*, *S. pneumoniae* and *S. typhi*. They were clinical isolates obtained from Usmanu Danfodiyo University Teaching Hospital, Sokoto. The cultures of the test bacteria were maintained on nutrient agar slants at 4°C. Their identities were further confirmed using standard biochemical tests (Chesebrough, 1982).

Extraction and fractionation of the plant material

Fractionation of the extracts was done by activity-guided fractionation using methanol in water (1:1), aqueous and n-hexane. Here, 50 g of the powdered *S. italica* leaves were extracted with 500 ml of methanol-water (1:1) at room temperature over night. The extract was filtered using Whatman No. 1 filter paper. The filtrate was partitioned in n-hexane (250 ml) and clarified by further filtration. Evaporation of the n-hexane to dryness was done in an oven at 45°C. A separate portion (50 g) of the powdered leaf was extracted with 500 ml distilled water at room temperature over night and filtered. The filtrate was also evaporated to dryness. The residue was reconstituted in sterilized distilled water and screened for antibacterial activity. Same procedure was followed using the powdered root (Isaac and Chinwe, 2001).

Phytochemical screening of the plant extracts

Test for alkaloids

A quantity (3 ml) of the concentrated leaf extracts was taken into a test tube and 1 ml of HCl was added. The mixture was heated gently for 20 min, cooled and filtered. The filtrate was used for the following tests: Firstly, two drops of Wagner's reagent were added to 1ml of the extract. The development of a reddish-brown precipitate was indicative of the presence of alkaloids in the extract. Secondly, two drops of Dragendorff's reagent were added to 1ml of the concentrated filtrate. The development of a creamy precipitate was indicative of the presence of alkaloids in the extracts. Same procedure was followed for the root extracts (Sofowora, 1985).

Test for saponins

Frothing test: Here, 2 ml of the leaf extracts contained in a test tube was vigorously shaken for two minutes. The presence of froth in the extract indicated the presence of saponins. On the other hand, for the emulsion test: Five drops of olive oil were added to 3 ml of the concentrated extract in a test tube and the mixture was vigorously shaken. Formation of a stable emulsion in the extract indicated the presence of saponins. Same treatment was given to the root extracts (Harborne, 1973).

Test for glycosides

A quantity (20 ml of 50% H₂SO₄) was added to 2 ml of the concentrated leaf extracts in a test tube. The mixture was heated in a water bath for 15 min. A quantity (10 ml) of Fehling's solution was then added and the mixture was boiled. Development of a brick-red precipitate indicated the presence of glycosides in the extracts. Same treatment was given to the root extracts (Trease and Evans, 1978).

Test for tannins

A quantity (4 ml of 5% FeCl₃) was added to 2 ml of the leaf extracts. Formation of a dark green precipitate indicated the presence of tannins. On the other hand, 4 ml of freshly-prepared 10% KOH was added to 4 ml of the concentrated leaf extract. Formation of a dirty-white precipitate indicated the presence of tannins in the extract. Same treatment was given to the root extracts (El-Olemyl et al., 1994).

Test for steroids

Here, 5 drops of concentrated H₂SO₄ were added to 1 ml of the leaf extract. Development of red colouration was indicative of a positive reaction. Similar procedure was followed for the root extract (Trease and Evans, 1978).

Test for flavonoids

Here, 1 ml of 10% NaOH was added to 3 ml of the leaf extract. Development of yellow colouration was indicative of a positive result. Same procedure was adopted using root extract (Trease and Evans, 1978).

Preparation of paper discs, extract concentrations and standardization of the test inoculum

Whatman No. 1 filter paper was punched using a sterile puncher into

Table 1. Phytochemical characteristics of *Senna italica* root and leaf (values in brackets) extracts.

Active compounds/solvents of plant extracts	Alkaloids	Tannins	Glycosides	Saponins	Steroids	Flavonoids
Methanol	+(+)	-(-)	-(-)	-(-)	+(+)	+(+)
N-hexane	+(+)	-(-)	-(-)	-(-)	+(+)	+(+)
Aqueous extracts	+(+)	-(-)	-(-)	-(-)	+(+)	+(+)

+ = Present; - = absent.

Table 2. Antibacterial activity of *Senna italica* leaf extracts.

Plant extract	Extract conc. (mg/ml)	Zone of inhibition (mm)				
		<i>S. aureus</i>	<i>Salmonella typhi</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Strept. pneumoniae</i>
N-hexane	30	11.60 ± 1.05	11.60 ± 1.05	16.00 ± 0.41	12.60 ± 0.65	14.00 ± 0.41
	60	12.60 ± 0.85	13.00 ± 1.64	22.00 ± 1.23	14.00 ± 1.23	16.00 ± 1.05
	90	16.00 ± 1.23	28.00 ± 0.82	29.00 ± 1.23	29.30 ± 0.75	29.00 ± 0.41
	120	20.00 ± 0.82	32.50 ± 0.50	32.50 ± 0.50	33.00 ± 1.64	31.25 ± 0.25
Tetracycline (Positive control)	10	24.20 ± 0.40	22.00 ± 1.05	28.00 ± 1.23	-	20.00 ± 0.82
	30	13.00 ± 1.46	11.50 ± 0.41	12.60 ± 0.65	14.00 ± 0.41	12.60 ± 0.65
Aqueous extract	60	22.30 ± 0.40	19.00 ± 0.41	14.00 ± 0.41	28.00 ± 1.23	20.00 ± 0.82
	90	28.00 ± 0.82	20.50 ± 0.50	29.00 ± 1.23	29.30 ± 0.75	29.00 ± 0.41
	120	32.50 ± 0.50	25.25 ± 0.25	32.50 ± 0.50	33.00 ± 1.64	31.25 ± 0.25
	Methanol in water (Negative control).	30%	-	-	-	-

- = No activity.

discs of 6mm diameter each. Twenty (20) pieces of the paper discs were soaked in 30, 60, 90 and 120 mg/ml of the leaf residue obtained and also the water extract for 24 h. The paper discs were then removed and dried at 45°C for 30 min. For the standardization of the test inoculums, pure cultures of the test organisms were inoculated onto nutrient broth (Oxoid, England) and incubated at 37°C for 24 h. They were then diluted using sterile nutrient broth to a density of 9.0×10^8 cfu/ml, which was equivalent to McFarland test tube number 4. The suspension was used to streak for confluent growth on the surface of nutrient agar plates using sterile swabs. Four of the paper discs prepared earlier were placed on the agar plates containing the swab of the test isolate. The plates were incubated invertedly at 37°C for 24 to 48 h for zones of inhibition. Similar treatment was given to the root extract (Vlientinck et al., 1995).

RESULTS AND DISCUSSION

World Health Organization (1991) defined a medicinal plant as 'any plant which, in one or more of its organs, contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The phytochemical screening of the *S. italica* root and leaf extracts revealed the presence of alkaloids, steroids and flavonoids while saponins, glycosides and tannins were not detected (Table 1). These compounds have been known to possess medicinal activities particularly antibacterial activity (Gronhaug et al., 2008; Kumar et al., 2009). The variation

in type of phytochemicals present in different solvents as shown in the results of phytochemical screening (Table 1) might be attributed to the ability of the solvent to dissolve into solution specific type of phytochemicals as reported by Kawo (2007), Yusha'u et al. (2008) and Kawo et al. (2009). This could have also explained the reason for the non-detection of saponins, glycosides and tannins in all the extracts screened in this study. Seasonal variations can affect the chemical composition of the plants and thus biological activity (WHO, 2003). The geographical location of a plant can affect its active constituents, which may be induced by many factors like climate, soil, propagation method, etc (Adoum et al., 1997). Time of collection of plant parts also affects its effectiveness (Odugbemi, 2008). All or a combination of the above mentioned factors could have contributed in the lack of antimicrobial activity observed in this study. The results of antibacterial activity indicated that both extracts were active against the five test bacteria with highest activities at the highest concentration (120 mg/ml) of all the extracts tested. Thus, inhibition zones of 20.0 ± 0.82 , 32.0 ± 0.50 , 32.0 ± 0.50 , 33.0 ± 1.64 and 31.25 ± 0.25 mm were recorded at the highest concentration of 120 mg/ml for the leaf extracts against *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa* and *S. pneumoniae* respectively (Table 2). Similar trend was observed for the root extracts (Table 3). These results strongly suggest that the

Table 3. Antibacterial activity of *Senna italica* root extracts.

Plant extract	Extract conc. (mg/ml)	Zone of inhibition (mm)				
		<i>S. aureus</i>	<i>Salmonella typhi</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Strept. pneumoniae</i>
N-hexane	30	29.0 ± 0.41	20.50 ± 0.50	11.50 ± 0.41	22.80 ± 0.60	-
	60	24.70 ± 1.20	24.70 ± 1.20	20.50 ± 0.50	24.00 ± 0.41	-
	90	38.5 ± 1.25	36.24 ± 0.25	22.80 ± 0.60	36.25 ± 0.25	-
	120	48.00 ± 0.82	38.5 ± 1.25	24.00 ± 0.41	48.00 ± 0.41	-
Tetracycline (Positive control)	10	25.25 ± 0.25	22.80 ± 0.60	28.00 ± 0.82	28.00 ± 0.82	19.00 ± 0.41
	30	24.70 ± 1.20				
Aqueous extract	60	36.24 ± 0.25				
	90	38.5 ± 1.25				
	120	48.00 ± 0.82				
	Methanol in water (Negative control)	30%	-	-	-	-

- = No activity.

components had a wide-broad spectrum antibacterial activity especially the fact that these organisms are also causative agents of other ailments in addition to the stomach disorder.

CONCLUSIONS AND RECOMMENDATIONS

The results obtained in the present study point to the pharmacological significance of *S. italica*; a fact that justifies the use of the plant in the treatment of some microbial diseases and infections as claimed by traditional herbalists. The results further suggest that the extracts of this plant contain bioactive elements; which could explain the rationale for the use of the plant in traditional medicine. However, it is recommended that further research be carried out in order to isolate and purify the bioactive constituents using various extraction solvents as isolation of pure compounds increases antimicrobial activity. On the other hand, indiscriminate and non-medical use of antibiotics, plant decoctions and concoctions should be minimized as this could contribute to the emergence of resistant bacterial strains.

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