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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *SENNA SIAMEA* (LAM) ON *PSEUDOMONAS AERUGINOSA*

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ABSTRACT
The anti-pseudomonal activities of aqueous, chloroform and ethanolic extracts of leaf of Senna siamea (Lam) were evaluated by agar disc diffusion method. This was with the aim of substantiating the ethnomedicinal use of the plant as anti-bacterial. All the extracts were not active to Ps. aeruginosa at the concentration levels of 100µg/disc and 200µg/disc, however the extracts were active at high concentration levels of 500µg/disc and 1000µ/disc revealing a dose – dependent antibacterial activity. Aqueous extract was the most active with zone diameter of inhibitions of 16mm and 30mm followed by ethanolic extract which had zones of inhibition of 10mm and 16mm and the least was chloroform extract which has zone diameter of inhibitions of 08mm and 14mm, all at 500µg/disc and 1000µg/disc concentrations respectively. Ciprofloxacin (oxoid) as positive control had zone diameter of inhibition of 35mm in all the test plates. The anti-psedomonal potential of the leaves of Senna siamea Lam leaf may be due to the phytochemical compounds present in the leaf extracts, which were found to be tannins, saponnins and steroids. However aqueous extract proved to be a more suitable candidate for use in the treatment of diseases cause by Pseudomonas aeruginosa. Hence, antimicrobial evaluation and biotechnological study of the whole plant are thus recommended.

Keywords: Antipseudomonal, Senna siamea, Phytochemicals. Screening, Pseudomonas aeruginosa

INTRODUCTION

A traditional claim have cited *Senna siamea* Lam (Kassod tree - English; Malga/marga - Hausa) to be used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, and is also used to reduce sugar level in the blood. It was also reported by Aliyu (2006) that *S. siamea* is ethnomedicinally used as laxative, blood cleaning agent, cure for digestive system and genitourinary disorders, herpes and rhinitis. Thus, it is necessary to further evaluate the pharmacological potential use of *S. siamea* leaves for the treatment of many other diseases.

Senna is an Arabian name and the herb was first brought into use by the Arabian physicians Serapion and Mesue. *Senna* is native to tropical Africa and cultivated in Egypt, Sudan and elsewhere, it is a medium size tree to 15 – 20cm tall, with a straight trunk up to 30cm in diameter, bole short, crown usually dense and rounded at first, later becoming irregular and spreading with dropping branches Bernand, 2005). *Senna siamea* leaves are locally used as antimalaria drug especially when decocted (Lose *et al.,* 2000). In traditional medicine, the fruit is used to charm away intestinal worms and to prevent convulsion in children (Alli – Smith, 2009).

In an attempt to rationally identify which pathogen to screen, *Pseudomonas aeruginosa* was epidemiologically identified as the hardiest bacterium that constitutes problems to researchers and clinicians. As literature showed, the hardy nature of *Ps aeruginosa* is exhibited even on the most active antimicrobials and antiseptics. It is also a notorious nosocomial pathogen capable of surviving for extended periods in almost any liquid environment. Moreover, on the average, 3% of person entering the hospital have *Ps aeruginosa* in their stools after staying for as little as 72hours in the hospital as reported by many authors (Jawetz *et al.* 1998; Hachem e*t al.* 2001; Wiblin, 1997).

It is therefore imperative to continue to screen suspected potential plants for a promising candidate against the organism. The aim of this research therefore, was to screen the leaf extracts of *Senna siamea* for bioactivity against *Pseudomonas aeruginosa.* This is with a view to finding out potent and efficacious plant extracts for use in the treatment of diseases caused by the bacterium.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Senna siamea (Kassod tree) was collected from the Botanical garden of the Department of Biological Sciences, Bayero University Kano, Nigeria and identified at the herbarium of the same department with the help of literature guide (Aliyu, 2006) and standard keys (Novak 1966).

Processing of Plant Materials

The leaves of *Senna siamea* were handpicked and washed thoroughly with distilled water. It was then air dried under shed at ambient room temperature. This was pounded into power by using a clean mortar and pestle. The powder was sieved to get a fine power.

Extraction Procedure
Five hundred grams (500g) of the air dried and grounded *Senna siamea* leaves was extracted by percolation with 400ml of 95 %ethanol at room temperature and kept for two weeks. The content was filtered by using Whatman No. 1 filter paper and the residue was discarded and later the extract was obtained following evaporation to dryness using rotary evaporator (R110) (Allex manufacturing limited) at 40^0C . The filtrate (Ethanol extract) "E" was partitioned further between water and chloroform as solvents in the appropriate ratio of 1:1 (50ml). The water-soluble fraction (H_20) was evaporated to dryness and chloroform-soluble fraction ($CHCl_3$) was kept to dry overnight at ambient room temperature. The residues were stored in a freezer at 4^oC until needed for use (Adoum *et al.,* 1997).

Preliminary Phytochemical Screening
Tests for Reducing sugar, tannin, steroid glycoside, resins, flavonoids, alkaloids and saponins was carried out as described by Brain and Turner (1975) and Cuilel (1994).

Preparation of Sensitivity Disc
Whatman No. 1 filter paper was punched using a paper puncher to obtain discs of 6.0mm in diameter. These were then placed in a sterile screw-capped Bijou bottles and sterilized in an oven using dry heat at 140^0C for 1 hour. The discs were allowed to cool until use. Stock solution of the plant extract was prepared in a screw capped bijou bottles using Dimethyl Sulphoxide (DMSO). One gram (1g) of each fraction was weighed on Metler balance (Model AE 160), and dissolved in 10ml of DMSO to arrive at 100,000µg/ml concentration of stock solution. From the stock solution, 0.1ml, 0.2ml and 0.5ml was added to 0.9ml, 0.8ml and 0.5ml of DMSO in test tubes to make 1ml each respectively. One hundred (100) sterilized discs were placed into each of the bottles to arrive at disc potencies of 100µg/disc 200µg/disc, 500µg/disc respectively. A. concentration of 1000µg/disc was also prepared for the bioassay.

Test Organism
The test organism used for the antimicrobial activity test was *Pseudomonas aeruginosa.* It was obtained from Murtala Mohammad Specialist Hospital (MMSH) as a clinical isolate from various urine samples of patients with urinary tract infections. Gram's staining and Biochemical test (Oxidase) were carried out to confirm the isolate, and it was grown on differential medium (MacConkey) (Cheesbrough, 2000). It was maintained in nutrient agar (Oxoid) slants throughout the period of the research. The slants were sub-cultured into a fresh slant fortnightly to maintain a young culture.

Preparation of Inoculum
This was achieved as described by Deeni and Hussain (1991). An overnight nutrient broth culture of the test organism was used to prepare an inoculum of about $3.3x10^6$cfu/ml. This was arrived at by appropriate dilution of the culture in 0.5% NaCl (w/v) to match the standard turbidity of 1% barium sulphate suspension (Mukhtar and Tukur, 2000), which marched the 0.5 Macfarland standard.

Bioassay Procedure
Agar diffusion method as descried by Kirby and Bauer (1966) and demonstrated by Mukhtar and Tukur (2000) was adopted. Nutrient agar plates were prepared and excess surface moisture in agar were removed for 10 minutes. The plates were aseptically inoculated with test organism by streaking method. With the aid of sterile pair of forcep, impregnated paper disc containing the extract of *Senna siamea* leaves at different concentrations (100µg/disc, 200µg/disc, 500µg/disc and 1000µg/disc) were arranged radially and pressed firmly to the inoculated agar surface to ensure even contact. Each disc was sufficiently spaced out and kept at least 15mm from the edge of the plate to prevent over lapping of zones. Positive and negative control discs containing standard antibiotic i.e. ciproflaxacin (30µg/disc) and only dimethysulfoxide (DMSO) were respectively also placed on the surface of the plates. The plates were incubated at 37^0C for 18hours in aerobic condition. Diameter of zone of inhibition was measured using meter rule and was recorded in millimeter (Mukhtar and Okafor, 2002).

RESULTS AND DISCUSSION
Table 1 shows the result of phytochemical screening carried out on the leaf of *Senna siamea* leaf extracts. Phytochemical compounds present were found to be reducing sugars and tannins in all the extracts, saponins were found to be present in only the aqueous extract, and resins in ethanolic extract only. Steroids were found to be present in both the ethanol and aqueous extracts, while flavonoids and alkaloids were not detected in all the extracts (Table 1). The presence of various phytochemicals in *S. siamea* extract and fractions have also been reported by Alli – smith (2009); Ahmed – Alizaga and Olayanju (2007). The variation in type of phytochemicals present in different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvents to dissolve into solution specific type of phytochemicals as reported by Yusha'u *et al.* (2008). The antibacterial activity of *S. senna* leaves could be due to phytochemicals present such as saponins, alkaloids, tannins etc, 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.

Tables 2, 3 and 4 show the antimicrobial activity of the ethanolic extract of *Senna siamea* (leaves) on *Ps. aeruginosa.* The organism was resistant to all the extracts at 100 and 200µg/disc concentrations, with antipseudomonal activity only exhibited at 500µg/disc and 1000µg/disc concentrations, for all the extracts.

The zone diameter of inhibitions of 10mm and 16mm; 08mm and 14mm and 16mm and 30mm for ethanolic, chloroform and aqueous extracts respectively reveal a dose – dependent antipseudomonal activity. The antibacterial activity of *S. senna* have been previously reported by Abo *et al.* 1999; Ayfer and Ozlem (2003); Elujoba *et al.* 1999, Kumar *et al.* (2006): and Ahmed – Alizaga and Olayanju, 2007), while the dose – dependent antibacterial activity of *S. senna* have been reported by Ahmed - Alizaga and Olayanju (2007). The result also revealed aqueous extract to possess higher antipseudomonal activity compared to other extracts; chloroform extract exhibited the least activity. The high antibacterial activity of aqueous extracts over the other extracts could be attributed to its possession of more phytochemical constituents which include tannins and saponins. Saponins were absent in ethanol and chloroform extract. The presence of tannins and saponins in an extract could increase its antibacterial activity as reported by Harbone (2000). The zone diameter of inhibition of the aqueous extract at 1,000 µg/disc (30mm) almost reach that of the positive control disc (ciprofloxacin) at 30µg/disc (35mm), which is an indication of its promise as a strong antipseudomonal agent.

Recently, the extraction of plant parts for antipseudomonal activity is given more attention due to the alarming discoveries of multiple drug resistance of *Pseudomonas aeruginosa* and other microbes as there are a large number of potentially medicinal plants worthy of investigation for antibiotic activity (Sandberg and Bruhn, 1979). Plants in Kano reported to have antipseudomonal activity include *Cyperus articulatus* (Usman, 2004); *Dodonea viscosa* (Halliru, 2007) and *G. senegalensis* (Alade, 1995).

Table 1: Phytochemical characteristics of leaf extract and fractions of *C. siamea*

	Solvents		
Phytochemicals	**Ethanol**	**Aqueous**	**Chloroform**
Saponins	-	+	-
Test for reducing sugar	+	+	+
Tannins	+	+	+
Resins	+	-	-
Flavonoids	-	-	-
Alkaloid	-	-	-
Steroids	+	+	-

Keys: + = present
- = absent

Table 2: Antibacterial activity of ethanolic extract of *C. siamea* on *Ps. aeruginosa*

	Concentration (µg/disc)				Ciprofloxacin (30µg/disc)
	1000	500	200	100	
Test organism	**- Zone of inhibition (mm)**				
Pseudomonas aeruginosa	16	10	00	00	35

Table 3: Antibacterial activity of chloroform fraction of *C. siamea* on *Ps. aeruginosa*

	Concentration (µg/disc)				Ciprofloxacin (30µg/disc)
	1000	500	200	100	
Test organism	**Zone of inhibition (mm)**				
Pseudomonas aeruginosa	14	8	00	00	35

Table 4: Antibacterial activity of aqueous (water) extract of *C. siamea* on *Ps. aeruginosa*

	Concentration (µg/disc)				Ciprofloxacin (30µg/disc)
	1000	500	200	100	
Test organism	**Zone of inhibition (mm)**				
Pseudomonas aeruginosa	30	16	00	00	35

CONCLUSION

From the result of this investigation, it could be concluded that the leaf aqueous extract of *S. siamea* could be suitable candidate in the preparation of drugs for the treatment of infections caused by *Ps. aeruginosa,* while the ethanol and chloroform extracts has shown to be least candidate in terms of activity. Reducing sugars, tannins, saponins and perhaps other bioactive principles such as resins could be attributed to the anti *pseudomonal* activity exhibited by the leaf extracts.

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