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Full Length Research Paper

Phytochemical and antimicrobial screening of the stem aqueous extract of *Anisopus mannii*

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Studies were conducted to determine the active chemical constituents of stem aqueous extract of *Anisopus mannii* by way of phytochemistry and to evaluate its *in vitro* antimicrobial activity, using the disc diffusion technique as described by the National Committee of Clinical Laboratory Standard (1993). Using standard phytochemical analysis procedures, results revealed the presence of saponins, alkaloids, flavonoids, glycosides, terpenes and steroids. Tannins and anthraquinones were not detected in the extract. The stem aqueous extract of *A. mannii* dose dependently inhibited the growth of *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Salmonella gallinarium, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* except for *Candida albicans* where the extract did not show any activity. The minimal inhibitory concentration (M.I.C) values were 25 mg/ml for *E. coli*, 50 mg/ml for *S. aureus, S. pyogenes, S. gallinarium, and P. aeruginosa* and 100 mg/ml for *K. pneumoniae*. The minimal bactericidal concentration (M.B.C) was 50 mg/ml for *E. coli, S. aureus, S. gallinarium,* and *P. aeruginosa*, 100 mg/ml for *S. pyogenes* and 200 mg/ml for *K. pneumoniae*. These results suggest that the stem aqueous extract of *A. mannii* could be promising in its potential usefulness for treatment of bacterial induced ailments.

Key words: Phytochemical, extract, antimicrobial, *Anisopus mannii*.

INTRODUCTION

Dependence on herbs as medicine in the treatment of diseases is still much practiced by a large proportion of the rural populace because of its availability and affordability. Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile. It is not only important to supplement modern drugs with effecttive local herbs for economic reasons but also for the fact that there is acute shortage of drugs (Sofowora, 1984). Investigations into the antimicrobial activity of some Nigerian medicinal plants have been carried out (Kudi et al., 1999; Ogundipe et al., 2000; Isaac and Chinwe, 2001; Dahiru et al., 2004; Sanni, 2007) with a view to authenticate their folkloric use (Locher et al., 1995; Williams, 1996).

The plant *Anisopus mannii* (Family Asclepiadaceae) is a glabrous twining shrub, strong climber with greenish flower in globose, lateral umbelliform cymes and horizontally opposed follicles 6 – 8 inch long and about half inch thick, tapering to a slightly hooked point at the apex (Hutchinson and Dalziel, 1963). It is known as 'Sakayau' or 'Kashe zaki' among the Hausas of the northern Nigeria, where a cold decoction of the stem is traditionally used as remedy for hyperglycemia. Despite the widespread use of this plant in this environment, neither its phytochemistry nor pharmacological effects has been evaluated and reported before to our know-ledge of survey of the literature.

The study, therefore, was designed to identify the active principles in the plant extract and to investigate the *in vitro* antimicrobial effect of the aqueous stem extract of the plant.

MATERIALS AND METHODS

Collection, identification and processing of plant material

Stems of *A. mannii* were collected from Kuwayange village in Damboa Local Government area of Borno state, Nigeria, in the month of

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Table 1. Phytochemistry of stem aqueous extract of *A. mannii*

TESTS	RESULT
Saponins	
(i) Frothing test	+
(ii) Emulsion test	+
Alkaloids	
(i) Meyer's test	+
(ii) Wagner's test	+
(iii) Draggendoff's test	+
Flavonoids	
(i) Ferric chloride test	+
(ii) Sodium hydro-oxide test	+
(iii) Lead acetate test	+
(iv) Pew test	–
Terpenes and Steroid	
(i) Lieberman- buchard's tests	+
(ii) Salkowski's test	+
Glycosides	
(i) General test	+
Tannins	
(i) Ferric chloride test	–
(ii) Potassium hydroxide test	–
Anthraquinones	
(i) Free anthraquinones	–
(ii) Combined anthraquinones	–

+: present; -not detected

January, 2008. The plant was identified and authenticated by Prof. S.S. Sanusi of Botany Department, University of Maiduguri, Borno state, Nigeria where a voucher specimen was deposited for reference. The stems were sorted, cleaned and air dried at room temperature for 2 weeks and then crushed to powder with a pestle and mortar.

Preparation of aqueous extract

About 100 g of the powdered sample was mixed with 500 ml of distilled water, in a conical flask. The mixture was stirred severally and covered overnight at room temperature and then filtered using Whatman filter paper, No 1. The filtrate was then evaporated to dryness using digital heating drying oven (DHG-9030A) preset at 50^{o}C and was later stored at 4^{o}C. The yield of the extract was 8.2% w/w.

Phytochemical analysis

The extract was evaluated for the presence of saponins, flavonoids, glycosides, tannins, alkaloids, terpenes and steroids using simple qualitative methods of Sofowora (1984) and Harborne (1993).

Micro-organism

The following bacteria clinical isolates were obtained from stock culture of pathological strains, preserved at the Veterinary Medicine Laboratory, Faculty of Veterinary Medicine, University of Maiduguri: *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Salmonella gallinarium, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Candida albicans.*

Antimicrobial activity

The disc diffusion method as described by national committee for clinical laboratory standards (1993) was used to determine the growth inhibition of bacteria by the plant extract. Discs containing different concentrations (125, 250 and 500 mg/ml respectively) of dissolved extract were prepared using sterile Whatmann filter paper No. 1, (6 mm in diameters). The discs were dried at 50^{o}C. Overnight cultures of each of the bacterial isolates was diluted with sterile normal saline to give inoculum size of 10^{6} cfu/ml. Nutrient agar medium was prepared, sterilized, cooled and poured into sterile Petri dishes to a depth of 4 mm (about 25 ml per plate) to solidify.

Pure cultures of the test organisms were used to inoculate the Petri dishes. This was done by spreading the inocula on the surface of the prepared nutrient agar plate using sterile cotton swabs which have been dipped in the diluted suspension of the organism. The discs were then aseptically placed evenly on the surface of the inoculation and gently pressed down to ensure contact using a pair of forceps. The plates were finally incubated at 37^{o}C for 18 – 24 h. Tetracycline (25 mg) (Greenfield Pharmaceutical Co. LTD, JIANG-SU Province, China) was included in each of the inoculated plates as positive control. Plates prepared using the same procedures without extract or antibiotic were equally set as negative control. The plates were examined after 24 h for clear zone of inhibition. Antibacterial activity by the extract was measured and recorded using a pair of calipers and compared to the standard antibiotics as in this study.

Determination of minimal inhibitory concentration (M.I.C) and minimal bactericidal concentration (M.B.C)

The method of Greenwood (1989) was used to determine the M.I.C and M.B.C of the extract. Five sterile test tubes were arranged in a test tube rack and 0.5 ml of sterile nutrient broth was pipetted into each test tube. Half ml of the crude extract containing 200 mg/ml was pipetted into each of the five tubes containing the broth. Thereafter, there was a serial dilution of the extract to obtain concentrations of 100, 50, 25, and 12.5 mg/ml respectively. The test organism (0.5 ml) was pipetted into each of the test tubes containing the mixture of the broth and extract and then finally incubated at 37^{o}C for 18 - 24 h. The M.I.C was recorded as the least concentration of plant extract that completely inhibited the growth of the test organism. The M.B.C was determined by sub culturing the contents of the tubes for 18 – 24 h to determine bactericidal activity. The M.B.C was demonstrated when no growth occurred on the sub cultured medium.

RESULTS AND DISCUSSION

The results of the phytochemical analysis show that saponins, flavonoids, alkaloids, glycosides, terpenes and steroids are present in the extract. Tannins and anthraquinones were not detected (Table 1).

The extract produced a dose dependent zone of inhibition in all the organisms tested except for *C. albicans* where the extract did not show any activity.

However, the effects observed were less than those produced by the standard agent (tetracycline) (Table 2). The minimal inhibitory concentration (M.I.C) of the plant extract was 25 mg/ml for *E. coli*, 50 mg/ml for *S. aureus,*

Table 2. Antimicrobial screening of stem aqueous extract of *Anisopus mannii.*

Extract Conc. (Mg/ml)	Zone of inhibition (mm)						
500	17	10	10	12	9	10	-
250	16	8	7	10	8	8	-
125	14	7	7	8	-	7	-
TCL(25)	28	30	25	30	34	23	-

- : no activity
TCL: tetracycline

Table 3. Minimal Inhibitory Concentration of stem aqueous extract of *Anisopus mannii*

Organisms	Concentration (mg/ml)				
	200	100	50	25	12.5
Escherichia coli	-	-	-	-	+
Staphylococcus aureus	-	-	-	+	+
Streptococcus pyogenes	-	-	-	+	+
Salmonella gallinarium	-	-	-	+	+
Klebsiella pneumoniae	-	-	+	+	+
Pseudomonas aeruginosa	-	-	-	+	+

-: Susceptible
+: Resistance

Table 4. Minimal bactericidal concentration of stem aqueous extract of *Anisopus mannii.*

Organisms	Concentration (mg/ml)				
	200	100	50	25	12.5
Escherichia coli	-	-	-	+	+
Staphylococcus aureus	-	-	-	+	+
Streptococcus pyogenes	-	-	+	+	+
Salmonella gallinarium	-	-	-	+	+
Klebsiella pneumoniae	-	+	+	+	+
Pseudomonas aeruginosa	-	-	-	+	+

-: Susceptible
+: Resistance

S. pyogenes, S. gallinarium and *P. aeruginosa* and 100 mg/ml for *K. pneumoniae* (Table 3). The minimal bactericidal concentration (M.B.C) of the extract was 50 mg/ml for *E. coli, S. aureus, S. gallinarium* and *P. aeruginosa*, 100 mg/ml for *S. pyogenes* and 200 mg/ml for *K. pneumoniae* (Table 4).

The organic constituents present in the extract have been reported to possess some medicinal uses (Blumgerter, 1989; Ogundipe et al., 1998; Abdulrahaman and Onyeyili, 2001). Flavonoids were reported to possess biological activity against microbes (Kinsella, 1993). Extract of the seeds of *Tetracarpidium conophorum* was reported to possess antimicrobial activity which was associated with its alkaloids, saponins, tannins, flavonoids, and glycosides contents (Isaac and Chinwe, 2001). Sanni (2007) reported that the antibacterial activity of the aqueous leaf extract of *Ocimum basilicum* was due to the presence of flavonoids in the plant. The antibacterial activity of *A. mannii* as recorded in this study may therefore be due mainly to the presence of flavonoids, saponins, glycosides and alkaloids in the extract. The non activity of the plant against *C. albicans* suggests that the plant extract

may not possess antifungal activity.

Conclusion

It has been concluded that the stem aqueous extract of *A. mannii* possess antibacterial activity. Its potential application in the treatment of bacterial infection would therefore be promising. Further work is required in order to isolate the active constituents of the plant responsible for the antibacterial activity.

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