Phytochemical Analysis and Antimicrobial Activity Screening of the Crude Extracts from the Aerial parts of *Tapinanthus globiferus* Linn

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Abstract

The aerial parts of Tapinanthus globiferus, belonging to the Loranthaceae family, which has various traditional medicinal applications, were studied. Phytochemical screening showed that they contain anthracene glycosides, alkaloids, saponins, tannins, aloes and sugars. The crude methanolic and chloroform extracts have growth inhibitory effects on Bacillus aureus, Providencia stautii, Escherichia coli, Pseudomonas aeruginosa, Staphylococus aureus and Klebsiella pneumonia. The minimum inhibitory concentration of the crude methanolic extract was determined for the various organisms whose growths were inhibited by the extract. However, the extract did not inhibit the growth of Candida albicans, Salmonella typhi and Aspergillus niger.

INTRODUCTION

The plant *Tapinanthus globiferus* belongs to the family called Loranthaceae (Linn)[1]. It grows parasitically on trees and shrubs. Nearly all the Loranthaceae family grow in the tropics. The plant was formerly named *Tapinanthus bawgwensis* but later renamed *Tapinanthus globiferus*. Plants in these genera have been reported to be of immense use in traditional medicinal applications, hence the need to investigate this particular member of the group[1]. For example, this plant's family has been reported to be particularly good for the treatment of the disorders of the female reproductive system especially when there is an emotional side to the problem. They have been reported to be good for treating young women who are emotionally tense and in treating them whenever they experience shock due to amenorrhea[1]. They are also used for nervous palpitations and infertility. The tea prepared from their herbs is reported to have a calming effect and serve as nervous tonic when there is tension that could lead to high blood pressure[1].

The same source has it that the herb tea from these plants acts on the vagus nerve of the heart and this helps to slow down the heart, reduces the blood pressure and also strengthens the capillary walls. It was reported that the decoction from this plant group is used for treating epilepsy and helps to calm down the electrical activities of the brain[1,2]. Again, report has it that cancer-like ulcers and growths, cracked feet, ulcerated legs and also malignant suppurating non-healing wounds, when treated by washing them with infusion from these plants, get healed.

In Nigeria and in the West African sub-region, the stem and bark infusion is used for the treatment of itching skin. The leaf decoction is drunk during pregnancy for unspecified purposes in northern Nigeria. In Guinea-Bissau, the plant is found useful in the treatment of various skin diseases. The leaf decoction is applied topically to rheumatic limbs, drunk as a remedy for chest disorders and even given as nasal drop for headache. The leaves are also given as inhaler for the treatment of colds. The paste made from the leaves is also placed on fractured limbs to enhance healing. The decoction from the leaves is used as a remedy for menstrual troubles and also for the treatment of asthma, leprosy and for the removal of guinea worm from the infected patients[2]. It is the numerous claims to the use of this plant in traditional medicinal applications that drew our attention to it.

EXPERIMENTAL

Plant materials

The aerial parts of *T. globiferus* were collected from Samaru in Zaria, Nigeria in the month of May, 2000 and air-dried. Voucher specimen of the plant was confirmed by Mallam Mohammed Musa and deposited in the Herbarium of the Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria, with Herbarium number 1052.

Extraction

The air-dried material was cut into smaller pieces and pulverised using a blender. Some quantity of the pulverised material (181.00g) was

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defatted using petroleum spirit (60 – 80°C boiling range) and the soxhlet apparatus. The defatted material was then successively and exhaustively extracted with chloroform and methanol respectively. The various extracts were concentrated *in vacuo* at 42°C using *rota vapor*. This afforded 1.16g (0.64%) of crude chloroform extract and 19.71g (10.89%) of crude methanol extract. These were then subjected to bioassay studies.

Phytochemical analysis of the plant

The pulverised plant sample was phytochemically screened using standard techniques[3] for carbohydrates, sugars, starch, cardiac glycosides, tannins, aloes and alkaloids. The results obtained in this screening are shown in Table 1.

Table 1. Results of the phytochemical screening on the crude sample.

Anthracene	Positive
Alkaloids	Positive
Aloes	Positive
Carbohydrates	Positive
Cardiac glycosides	Negative
Glycosides	Positive
Reducing sugars	Positive
Saponins	Positive
Starch (soluble)	Negative
Tannins	Positive
pentose	Positive

Biossay analysis of the plant

Anti-bacterial screening

Standard strains of *Klebsiella pneumonia, Bacillus aureus, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Providencia Stautii* and *Pseudomonas aeruginosa* were collected from the Microbiology Department, Ahmadu Bello University, Zaria, Nigeria. All cultures were tested for purity. The inocula were prepared by inoculating the stock culture into freshly prepared nutrient broth (Oxoid) and incubating aerobically at 37°C for six hours. Stock solutions of the plant extracts were prepared by dissolving 1.0g of each extract in 2.5 ml of dimethyl sulphoxide (DMSO) and for each of the extracts, concentrations of 50 µg/ml, 30 µg/ml, 20 µg/ml and 10 µg/ml were prepared. One millilitre aliquot of each of the solutions thus prepared was transferred into a sterile glass evaporating dish. This was followed by the addition of a sufficient number of sterile filter paper (Whattman No. 1) discs, to completely absorb the 1 ml aliquot. The paper discs now impregnated with the extracts were dried in an oven at 55°C.

Freshly prepared Muller – Hinton agar (Oxoid) plates were inoculated by placing a loopful of the inoculum into the middle of each plate and cross-streaking with wire loop[4-6]. Paper discs impregnated with each aliquot of the crude extracts were placed asceptically and pressed firmly on the surface of an inoculated agar plate. Control experiments were set up using paper discs soaked in pure DMSO. All the plates were incubated aerobically at 37°C for 24 hours after which they were examined for the zones of inhibition of growth[7]. The observed zones of inhibition of growth were measured and recorded in millimeters of their diametrical section as shown in Table 2.

Test organism	mm zones of inhibition measured at µg/ml of the test extract concentration of						
	$5x10^4$	$3x10^4$	$2x10^4$	$1x10^4$	$5x10^3$	0.0 (control)	
Bacillus aureus	15	11	10	8	0	0	
Providencia stautii	12	10	9	8	6	0	
Escherichia coli	10	8	0	0	0	0	
Pseudomonas aeruginosa	15	10	9	0	0	0	
Staphylococcus aureus	15	12	10	8	6	0	
Klebsiella pneumoniae	10	8	0	0	0	0	
Salmonella typhi	-	-	-	-	-	-	
Aspergillus niger	-	-	-	-	-	-	
Candida albicans	-	-	-	-	-	-	

Table 2. Results of the anti-microbial screening of the methanol extract.

Fungicidal activity test

Fresh pure isolates of *Candida albicans*, *Aspergillus niger* and *Salmonella typhi* were obtained from the Microbiology Department of Ahmadu Bello University, Zaria. The fungal cultures were maintained on slants of sabourd dextrose agar. The paper disc method was used in this process and the steps involved were the same as those described above for antibacterial screening except that the medium used was potato dextrose agar. However, the cultures and the control experiments were left in the incubator for 72 hours at 27°C. Then the plates were examined and the diameters of any zones of inhibition were measured and recorded in millimeters(Table 2).

Determination of the minimum inhibitory concentration (m.i.c.)

The tube dilution method[8,9] was employed in this determination. In a set of five sterile 7.62 cm by 1.27 cm test tubes, and for each extract, 1.0 ml of the prepared broth was transferred into each of the test tubes and 1.0 ml of the 400 mg/ml standard solution was transferred in the first test tube to give a concentration of 299 mg/ml. This was diluted out serially by the dilution method to give graded concentrations (in μg/ml) of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 respectively. A loopful from the culture which showed zone of inhibition was introduced into each of the test tubes which was then capped and left at room temperature for 72 hours. The tubes were then examined for the presence or absence of growth/turbidity. The lowest concentration inhibiting growth was taken as the m.i.c. and this was noted and recorded for the various microbes(Table 3).

RESULTS AND DISCUSSION

The phytochemical screening of the aerial parts of *Tapinanthus globiferus* showed the presence of carbohydrates, sugars, pentose, saponin glycosides, tannins, aloes and alkaloids as in Table 1. It is suggested that the tannin content of this plant is responsible for its claimed efficacy in the treatment of wounds and burns in Zaria.

Table 2 shows the result of the antibacterial screening recorded in millimeters of the diametrical sections of the respective zones of inhibition whereas Table 3 is the result of the m.i.c., recorded for the microbes whose growth had been inhibited by the respective extracts. The results showed that the methanol extract inhibited the growth of *B. aureus*, *P. stautii*, *E. coli*, *P. aeroginosa*, and *K. pneumonia* but did not inhibit the growth of *S. typhi*, *A. niger* and *C. albicans*. Equally important is the fact that the zones of inhibition produced by the methanol extract on these organisms compared fairly well with the zones of inhibition produced by gentamycin and rifanpicin[10], which are regular drugs used internationally for the treatment of diseases associated with these microbes. In this work by Machido and Ado, it was reported that gentamycin produced an inhibition zone of 22 mm at a concentration of 10μg/ml on the microorganism *K. pneumonia* whereas the methanol extract of this plant produced an inhibition zone of 10 mm at a concentration of 0.5μg/ml.

Table 3. m.i.c. measurements of the methanol extract.

Test organism	Concentration of extract in μg/ml			
	5×10^4	1.25×10^4	0.31×10^4	0.07×10^4
Bacillus aureus	- D[1]	+	++	+++
Providencia stautii	- E[1]	+	++	+++
Escherichia coli	- B[1]	+	++	+++
Pseudomonas areoginosa	-	- C[2]	++	+++
Staphylococcus aureys	-	-	- E[3]	+
Klebsiella pneumoniae	- B[1]	+	++	+++

- = not turbid; + = slightly turbid; ++ = moderately turbid ; +++ = highly turbid; D, E, B, C, & E are extract concentrations yielding minimum inhibition

Again the m.i.c. results show that the methanol extract of this plant is not just bacteristatic with respect to the organisms under study, but it is also bactericidal. The plant is used locally for the treatment of chest pains among others and this result really provides a scientific basis for the folk medicinal properties of the plant. However, the extract principles responsible for these activities

have not been elucidated and efforts are being directed in this direction.

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