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Preliminary Phytochemistry, Antibacterial and Antifungal Properties of extracts of *Asystasia gangetica* Linn T. Anderson grown in Nigeria

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ABSTRACT

The hexane, ethylacetate and methanol extracts obtained from the whole plant of Asystasia gangetica were evaluated invitro to determine inhibition of human pathogenic microorganisms made up of six bacteria and six fungi. The crude extracts inhibited the growth of twelve test organisms to different degrees. All the bacteria strains were sensitive to all the extracts at concentration ranging from 50 to 200mg/mL using the agar diffusion pour plate method. The inhibition of these test organisms were concentration dependent, activity being higher at higher concentrations of all the three extracts. The extracts showed higher antifungal properties on Candida albicans, Penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum with activity comparable to that of the reference drug, Tioconazole. Preliminary phytochemical investigation of the extracts revealed the presence of saponins, reducing sugar, steroids, glycosides, flavonoids and anthraquinones.

Keywords: *A. gangetica,* bioactivity, phytochemical screening, agar diffusion method.

INTRODUCTION

Asystasia comprises about 50 species, and is distributed in tropics of the old world, with about 30 species in tropical Africa [1,2,3]. Asystasia *gangetica* (L) T. Anderson (Acanthaceae) is a fast growing, spreading, perennial herb, with usually ascending, branched, quadrangular stem up to 2 m long, often rooting at the lower nodes [3,4,]. It is herbaceous groundcover that grows from 300-600mm in height. It has green, oval-shaped leaves with white-cream coloured flower with purple markings and the fruit is a club shaped capsule, splitting from tip to base [4]. It is a native

in tropical Africa, Arabia and tropical Asia, but has been introduced in many other tropical regions where it often naturalized [2]. It is widely distributed in Nigeria and throughout the world [5,6]. The plant is used in ethnomedicine for the treatment of heart pains, stomach pains, rheumatism, as vermifuge [7], anthelmintic [8]; while in Nigeria, the leaves are popularly used in the treatment of asthma [9]. The phytochemical analysis of the *A. gangetica* has not been reported previously. The leaves have been shown to contain large amounts of proteins; as well as amino acids, minerals, carbohydrate, lipids and fibre [10]. The acclaimed effectiveness of this plant in traditional medicine as anti-inflammatory, anthelmintic and also its bronchospasmolytic property [8,11] enhanced our interest to report the phytochemical and antimicrobial properties of *Asystasia gangetica* L.

Ganges Primrose

MATERIALS AND METHODS

Collection and authentication of the plant material.

The whole plant material of *Asystasia gangetica* was collected on from Ibadan, Oyo State of Nigeria, November 2009. Botanical identification and authentication was done by Mr. A.W. Ekundayo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen (FHI 108798) was deposited.

Preparation of plant extracts

Fresh whole plant of *Asystasia gagentica* was air-dried and weighed (990g). The dried material was successively extracted in hexane, ethylacetate and methanol for 10 days respectively using cold extraction method. The resultant hexane (6g), ethylacetate (5g) and methanol (8g) extracts were obtained by evaporation and stored in the refrigerator for further use.

Phytochemical studies
The Preliminary phytochemical screening of the hexane, ethylacetate and methanol extracts of *A. gangetica* was done using standard procedures [12,13,14,15,16].

Antimicrobial Assay
Microorganisms: Cultures of six human pathogenic bacteria made up of four gram negative and two gram positive were used for the antibacterial assay. These were; *Salmonella typhii* (UCH 4801), *Escherica coli* (UCH 00260), *Pseudomonas aeruginosa* (UCH 1102) and *Klebsiellae pneumonae* (UCH 2894) belongs to the gram-negative and *Bacillus subtilis* (UCH 74230) while *Staphylococcus aureus* (UCH 2473) belongs to the gram-positive. For the Antifungal assay, six fungi were also utilized. These were; *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum.* All the microorganisms used were clinical strains from the Medical Microbiology (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria.

Media: Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethylacetate and methanol were also used in solubilizing the extracts and as negative controls in the assays.

Antimicrobial agents: Gentamycin (10 µg/mL) and Tioconazole (0.7 mg/mL) were included as standard reference drugs in the study.

Antimicrobial activity determination
Agar diffusion-pour plate method (bacteria): An overnight culture of each organism was prepared by taken two wireloop of the organism from the stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for 18-24hr at 37^{o}C. From overnight culture,

0.1 mL of each organism was taken and put into the 9.9mL of sterile distilled water to obtained 10^{-2} inoculum concentration of the organism.

From the diluted organism (10^{-2}), 0.2mL was taken into the prepared sterile nutrient agar cooled to about 40-45^{o}C, then poured into sterile Petri dishes and allowed to solidify for about 45-60min. Using a sterile cork-borer of 8mm diameter, the wells were made according to the number of the test tubes for the experiment. For this work 8 wells were made. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24hr at 37^{o}C [18,19].

Agar diffusion-surface plate method (fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and solidified properly. 0.2mL of the 10^{-2} inoculum concentration of the organism was spread on the surface of the agar using a sterile Petri-dish cover to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8mm diameter. The graded concentrations of the extracts were put into the

including the controls. All the plates were left on the bench for 2hr to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25^{o}C for 72hr [17,18].

RESULTS AND DISCUSSION

The results of the phytochemical screening of the hexane, ethylacetate and methanol extracts of the whole plant are presented in Table 1. Preliminary phytochemical screening of hexane extract revealed the presence of reducing sugars, steroids, glycosides, flavonoids and anthraquinones while saponins, tannins and alkaloids were absent in the extract. Ethylacetate and methanol extracts of *A. gangetica* whole plant showed the presence of saponins, reducing sugars, steroids, glycosides, flavonoids and anthraquinones, while tannins and alkaloids were absent in both extracts.

Table 1: Phytochemical constituents of the hexane, ethylacetate and methanol extracts of *Asystasia gangetica*(whole plant)

Secondary metabolites	Extracts (whole plant)		
	Hexane	Ethylacetate	Methanol
Alkaloids	–	–	–
Saponins	–	+	+
Tannins	–	–	–
Reducing sugars	+	+	+
Steroids	+	+	–
Glycosides	+	+	+
Flavonoids	+	+	+
Anthraquinones	+	+	+

- *Absent, + Present*

The three extracts (hexane, ethylacetate and methanol extracts) inhibited the growth of six organisms to different degrees. All the bacteria strains were sensitive to all the three extracts at concentrations ranging from 25 to 200 mg/mL (Table 2), except *Salmonella typhii* and *Staphylococcus aureus* which showed sensitivity on hexane and ethylacetate extracts at higher concentrations of 100 and 200 mg/mL, while methanol extract inhibited the growth of *Klebsiella pneumonae* and *Salmonella typhii* (gram negative) also only at concentrations of 100 and 200 mg/mL.

All the extracts showed no inhibition against the growth of these organisms at lower concentrations of 6.25 and 12.5 mg/mL, except hexane and ethylacetate extracts which showed minimum inhibition on *Pseudomonas aeruginosa* at concentration of 12.5 mg/mL. Meanwhile, the antibacterial properties of hexane, ethylacetate and methanol extracts on the organisms were concentration dependent, activity being higher at higher concentrations of the extracts.

Table 2: Antibacterial activities of the hexane, ethylacetate and methanol extracts of *Asystasia gangetica* (whole plant)

Extracts	Extract conc/Ref./ Control (mg/ml)	Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm)					
		S.a	*E.coli*	*B.sub*	*Ps.a*	*Kleb*	*Sal.*
Hexane	6.25	_	_	_	_	_	_
	12.5	_	_	_	10	_	_
	25	_	_	_	12	_	_
	50	_	10	10	14	_	_
	100	10	12	14	18	12	10
	200	12	14	16	20	14	13
	Hexane	_	_	_	_	_	_
	Gentamycin	38	34	34	36	32	34
Ethylacetate	6.25	_	_	_	_	_	_
	12.5	_	_	_	10	_	_
	25	_	_	_	12	_	_
	50	_	10	10	14	10	_
	100	10	12	12	16	12	10
	200	14	14	14	18	14	12
	Ethylacetate	_	_	_	_	_	_
	Gentamycin	36	34	34	36	34	34
Methanol	6.25	_	_	_	_	_	_
	12.5	_	_	_	_	_	_
	25	_	10	10	_	_	_
	50	10	12	12	10	_	_
	100	12	14	14	12	10	10
	200	14	16	17	15	12	13
	Methanol	_	_	_	_	_	_
	Gentamycin	36	34	36	34	34	36

S.a	*Staphylococcus aureus*
E.coli	*Escherichia coli*
B.sub	*Bacillus subtilis*
Ps.a	*Pseudomonas aeruginosa*
Kleb	*Klebsiellae pneumonae*
Sal	*Salmonellae typhii*
C.a	*Candidas albicans*
A.n	*Aspergillus niger*
Rhiz	*Rhizopus stolon*
Pen	*Penicillum notatum*
T.r	*Tricophyton rubrum*
E.f.	*Epidermophyton floccosum*

The result of the antifungal activities of the hexane, ethylacetate and methanol extracts at concentrations ranging from 6.25 to 200 mg/mL is presented in Table 3. Six fungi were used in the study: *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum.* The hexane, ethylacetate and methanol extracts of *A. gangetica* exhibited higher antifungal properties on the test fungi with activity comparable to that of the reference drug, tioconazole against *Tricophyton rubrum* and

Epidermophyton floccosum. Further, the three extracts inhibited the growth of the other four fungi (*Candida albicans, Aspergillus niger, Rhizopus stolon* and *Penicillum notatum*) at concentrations between 50 to 200mg/ml but were inactive to all the six fungi at lower concentrations of 6.25 and 12.5mg/ml.

Table 3: Anfungal activities of the hexane, ethylacetate and methanol extracts of *Asystasia gangetica* (whole plant)

Extracts	Extract conc/Ref./ Control (mg/ml)	Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm)					
		C.a	A.n	Rhiz	Pen	T.r	E.f
Hexane	6.25	_	_	_	_	_	_
	12.5	_	_	_	_	_	_
	25	_	_	_	_	10	10
	50	10	16	_	10	14	12
	100	14	12	10	14	16	14
	200	18	16	14	18	20	16
	Hexane	_	_	_	_	_	_
	Tioconazole	26	24	22	26	24	22
Ethylacetate	6.25	_	_	_	_	_	_
	12.5	_	_	_	_	_	_
	25	_	_	_	10	10	10
	50	10	10	_	12	12	12
	100	14	12	10	14	16	14
	200	16	14	12	16	20	18
	Ethylacetate	_	_	_	_	_	_
	Tioconazole	26	24	23	25	23	24
Methanol	6.25	_	_	_	_	_	_
	12.5	_	_	_	_	_	_
	25	_	_	_	10	10	11
	50	10	10	10	12	12	14
	100	14	12	12	14	15	17
	200	17	14	14	16	18	20
	Methanol	_	_	_	_	_	_
	Tioconazole	26	23	26	23	21	22

S.a *Staphylococcus aureus*
E.coli *Escherichia coli*
B.sub *Bacillus subtilis*
Ps.a *Pseudomonas aeruginosa*
Kleb *Klebsiellae pneumonae*
Sal *Salmonellae typhii*
C.a *Candidas albicans*
A.n *Aspergillus niger*
Rhiz *Rhizopus stolon*
Pen *Penicillum notatum*
T.r *Tricophyton rubrum*
E.f. *Epidermophyton floccosum*

CONCLUSION

The Phytochemical screening of the extracts revealed the presence of steroids, glycosides, flavonoids, reducing sugars and anthraquinones. The antibacterial and antifungal activities of hexane, ethylacetate and methanol extracts of whole plant of *A. gangetica* further confirm the use of the plant in Africa traditional medicine for the treatment of heart pains, stomach pains, rheumatism, as vermifuge, anthelmintic, antiasthmatic, bronchospasmolytic and anti-inflammatory [1,3,8,9,10,11]. The antifungal activities of the extracts also suggest the use of the plant for the treatment of fungal diseases like skin and mouth disorders in addition to the medicinal uses cited in the literature [1,3,8,9,10,11]. The research is still in progress on bioactivity guided isolation and structural elucidation of the bioactive compound(s) responsible for the observed pharmacological activities.

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