Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (2011), 19 (2): 236-240 ISSN 0794-5698

Phytochemical and Antibacterial Properties of Leaf Extracts of *Ipomoea asarifolia*

***[1]M.S. Aliyu, [2] U. Lawal, [1] M.B. Tijjani, [1]M.H.I. Doko, [1]I. Garba, [1]H.A. Kokya, [1]S.A. Ado, [3]U.A. Hanwa and [4]M.M. Ibrahim**

[1]Department of Microbiology, A.B.U. Zaria
[2]Department of Biological Sciences, Umar Musa Yar'adua University, Katsina
[3]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria
[4]Department of Pharrmacognosy and Drug Development Ahmadu Bello University, Zaria
[*Corresponding author's e-mail: msaliyuzra@gmail.com, Phone no. +2348022360774]

ABSTRACT: The antibacterial potency of aqueous and methanol extracts of *Ipomoea asarifolia* leaves; a plant widely used by traditional medical practitioners in Nigeria was determined *in vitro* against three bacterial pathogens (*Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*) by agar well diffusion method. The pattern of inhibition varied with the extracts and the organisms tested. Both the aqueous and methanol extracts were potent on *E. coli* and *S. aureus* with maximum zone of growth inhibition of 21mm and 20mm at 200mg/ml respectively *Pseudomonas aeruginosa* was resistant to both extracts at all the concentrations tested. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against *E. coli* for aqueous extract were 50mg/ml and 100mg/ml respectively; and 100mg/ml and 200mg/ml for methanol extract respectively. Similarly, The MIC and MBC against *S. aureus* for aqueous extract were 100mg/ml and 200mg/ml; 200mg/ml for the methanol extract. Preliminary phytochemical screening reveal the presence of anthraquinones, saponins and tannins in both aqueous and methanol extracts; triterpenes, flavonoids and glycosides were found only in the aqueous extract while alkaloids were found only in the methanol extract. However, carbohydrate and steroids were absent in both extracts. The spectra of activities shown by the extracts could be attributed to the presence of these phytochemicals which signifies the potential of *I. asarifolia* as a source of therapeutic agents. These findings therefore, justify the traditional medicinal use of the plant.

Keywords: *Ipomoea asarifolia,* phytochemical, antibacterial potency, bacterial pathogens

INTRODUCTION

The use of plants for medicinal purposes is an age old tradition in Africa Asia and Latin America (Bibitha *et. al.*, 2002; Karou, *et al.*, 2006).The effectiveness of these medicinal plants is repeatedly validated in the laboratory. Roughly 80% of plants selected for analysis on the basis of ethnomedicinal information have demonstrated significant pharmacological activity (Fatope, 2001). Medicinal plants are cheap and renewable source of pharmacologically active substances. Approximately 20% of the plants in the world have been subjected to pharmacological and/or biological evaluation and a substantial number of new antibiotics introduced in to the market are obtained from natural or semi synthetic sources (Mothana and Lindequist, 2005).

Ipomoea asarifolia (*Convolvulaceae*) is a glabrous succulent perennial plant trailing on the ground. It is found throughout West Africa and is a common weed of hydromorphic soils, low lying and inland valleys, streams and river banks. In Nigeria, the traditional names include "*Duman kada*" in Hausa and "*Gboro ayaba*" in Yoruba (Jegede *et. al.*, 2009). In Senegal, the plant is used for various gynaecological purposes; urinary problems during pregnancy; haemorrhage, as an ecbolic and abortifacient, also in general for wound dressing and for the treatment of opthalmia, neuralgia, headache, arthritic pains and stomach ache while in Ivory Coast, pulped-up leafy stem are mixed with citron and water and taken as an ecbolic. A leaf decoction is usually taken internally as a wash for feverish chills and rheumatic pains. The leaves are used to treat dysmenorrhoea (painful menstruation) in the middle belt region of Nigeria while in northern Nigeria, the leaf poultice (moist substance applied to injury) is applied to guinea worm sores while

the face is steamed over a hot decoction of the plant along with husks of bulrush millet; a leaf poultice is applied to the guinea worm sores and the flowers are boiled with beans and eaten as a remedy for syphilis (Burkil, 1985; Jegede *et. al.*, 2009). The present study therefore, reports the results of the phytochemical screening of the leaves of *I. asarifolia* and the antibacterial potency of the leaves extract.

METHODOLOGY

Collection and identification of Plant Materials

Whole fresh plant growing wild was collected in Zaria in September, 2009. The plant was authenticated at the herbarium unit of the Department of Biological Sciences, A.B.U., Zaria (voucher no. 1911).

Leaves were air dried at room temperature for 21 days to a constant weight. The dried leaves were pulverized to coarse powder using mortar and pestle and sieved with 20 mesh (British standard). The fine powder was stored for further studies.

Extraction

The extraction methods described by Harborne (1973) and Aliyu *et. al.*, (2009) were adopted. Eighty five grams (85g) of the powdered plant material was soaked in water and methanol respectively. The mixture of each solvent was agitated in a mechanical shaker over night, filtered and concentrated using water bath and transferred to a Soxhlet apparatus, the filtrate was evaporated and the residues were used for phytochemical analysis and bioassay.

Phytochemical Analysis

The methods described by Cannel (2000) and Hassan *et. al.*, (2004) were used for the phytochemical screening.

Screening for Antibacterial Activity

Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. The susceptibilities of the test organisms to the plant extract were assayed as described by Aliyu, *et al.*, (2009). Briefly, the test organisms from growth on nutrient agar incubated at 37 ^{o}C for 18hr were suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 (10^{8} cells/ml) McFarland standard. The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates (90mm in diameter) using sterile cotton swab. Six millimeter diameter wells were punched using cork borer in agar and filled with the desired concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the aqueous and methanol extracts. Commercial antibiotic (Ciprofloxacin 30µg) was used as reference standard to determine the sensitivity of the isolates. Disc was directly placed onto the bacterial culture. The plates were allowed to stand for 5 hours at room temperature for extract to diffuse into the agar and then incubated at 37^{o}C over night. Antibacterial activities were evaluated by measuring inhibition zone diameters. The entire test was conducted in duplicate.

The minimum inhibitory concentration was determined according to the National Committee for Clinical Standard (1999). Each extract from aqueous and methanol was separately dissolved in sterile distilled water and 2ml of sterile Mueller Hinton broth was transferred into a set of 5 tubes and 2ml of each concentration (400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml) of the extracts was added to the test tubes respectively to obtain final concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively. The test organism was inoculated into the labeled tube except the control; the tubes were incubated at 37^{o}C for 18 h. The MIC was taken as the lowest concentration that prevented visible growth. The above procedure was repeated for each of the test organisms.

The minimum bactericidal concentration was determined according to the National Committee for Clinical Standard (1999). From the test tubes used in the determination of MIC, the tubes that showed no visible growth were sub cultured onto freshly prepared Mueller Hinton agar and incubated at 37^{0}C for 48. The least concentration at which the organisms did not recover and grow was taken as the MBC.

RESULTS

Table 1 shows the results of Preliminary phytochemical screening of *I. asarifolia* leaf extracts. Anthraquinones, saponins and tannins were found in both aqueous and methanol

extracts; triterpenes, flavonoids and glycosides were found only in the aqueous extract while alkaloids were found only in the methanol extract. However, carbohydrate and steroids were absent in both extracts.

Table 1: Phytochemical Profile of Aqueous and Methanol Leaf Extracts of *I. asarifolia.*

Phytochemical Constituent	Aqueous Extract	Methanol Extract
Carbohydrate	_	_
Anthraquinones	+	+
Saponins	+	+
Steroids	_	_
Triterpenes	+	_
Flavonoids	+	_
Tannins	+	+
Alkaloids	_	+
Glycosides	+	_

+ = Present - =absent`

Antibacterial assay of aqueous extract of *I. asarifolia* leaves revealed that the aqueous extract possessed antibacterial activity against two of the test organisms. The largest zone of inhibition was observed at 200mg/ml against *E. coli* (21.0mm) followed by *S. aureus* (20.0). However, *P. aeruginosa* was resistant to the extract (6.0mm) at all the concentrations tested. The observed antibacterial activities of the aqueous extract are presented in Table 2.

Table 2: Diameter of Zones of Inhibition (mm) by *I. asarifolia* Aqueous Leaf Extract against the Bacteria

Test organisms	Zones of inhibition (mm)/concentration (mg/ml)				
	200	**100**	**50**	**25**	**12.5**
E. coli	21.0	17.5	15.5	6.0	6.0
S. aureus	20.0	18.0	6.0	6.0	6.0
P. aeruginosa	6.0	6.0	6.0	6.0	6.0

Table 3 shows the inhibitory effect of methanol extract of *I. asarifolia* leaves. The extract showed various inhibitory effects against *E. coli* (17.5mm) at 200mg/ml and *S. aureus* (14.0mm) at 200mg/ml.

Results of the MIC and MBC are presented in Table 4. The MICs of the aqueous and methanol extracts against *S. aureus* were 100mg/ml and 200mg/ml respectively while *E. coli* had MIC values of 50mg/ml and 100mg/ml for aqueous and methanol extracts respectively. Higher MBC of 200 mg/ml was observed against *S. aureus* for both aqueous and methanol; however, *E. coli* had MBC values of 100mg/ml and 200mg/ml for aqueous and methanol extracts respectively.

Table 3: Diameter of Zones of Inhibition (mm) by *I. asarifolia* Methanol Leaf Extract against the Bacteria

Test organisms	Zones of inhibition (mm)/concentration (mg/ml)				
	200	**100**	**50**	**25**	**12.5**
E. coli	17.5	16.0	6.0	6.0	6.0
S. aureus	14.0	6.0	6.0	6.0	6.0
P. aeruginosa	6.0	6.0	6.0	6.0	6.0

Table 4: MIC and MBC of *I. asarifolia* leaf extract against the test organisms ($mgml^{-1}$)

Test organism	MIC aqueous	methanol	MBC aqueous	methanol
E. coli	50		100	
	100		200	
S. aureus	100		200	
	200		200	

DISCUSSION

Antibacterial evaluation of aqueous and methanol leave extracts of *I. asarifolia* revealed a significant antibacterial potency against the susceptible test organisms. Both the aqueous and methanol extracts were more potent on gram-negative *Escherichia coli* with maximum zone of growth inhibition of 21mm, at 200mg/ml. Although gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents (Ndukwe *et. al.*, 2005), however, impressive activity against this gram-negative bacterium was observed. *Escherichia* coli is incriminated in gastrointestinal and urinary tract infections; the susceptibility of *E. coli* to the extracts is an indication of the therapeutic potentials of these extracts against such diseases. The aqueous and methanol extracts have an appreciable potency against *S. aureus*. *Staphylococcus aureus* causes skin and soft tissue infections, thus, the potency of the extracts on the

organism justifies the folkloric use of the plant in the treatment of wounds and guinea worm sores. There was no observed inhibitory effect on *Pseudomonas aeruginosa* by all the extracts; this organism is resistant to plant extracts as reported by Mukhtar and Tukur, (2001) and Aliyu *et. al.* (2009). *Pseudomonas aeruginosa* has been reported to have possibly developed resistance to most antibiotics even before their discovery (Mukhtar and Tukur, 2001). MIC values of 50 mg/ml and 100 mg/ml were recorded against *E. coli* for aqueous and methanol extracts respectively while 100 mg/ml and 200 mg/ml were recorded against *S. aureus* for aqueous and methanol extracts respectively. MIC and MBC values of 200 mg/ml demonstrated by the methanol extract especially on *S. aureus* is an indication that the phytoconstituents have bactericidal potential.

Plants with pharmacologicaly active metabolites such as saponins, flavonoids, tannins, anthraquinones, alkaloids and triterpenes have been found *in vitro* to have antimicrobial properties (Cowan, 2002; Sibanda and Okoh, 2007; Aliyu *et. al.*2009). The antibacterial activity of *I. asarifolia* could be attributable to the various phytochemicals detected in its extracts. Saponins may act by damaging cell membranes causing leakage of cellular materials, ultimately leading to cell death (Mshvildadze *et. al.*, 2000). Triterpenes are synthesized from acetate units which share origins with fatty acids. Their tremendous activities against bacteria have been reported (Ahmed *et. al*., 1993; Barre *et. al*., 1997; Amaral *et. al*., 1998). Thus, the spectra of activity displayed by the crude extracts can be explained by the complementary effects of the secondary metabolites present or due to synergism. The purified components may have even more potency with respect to inhibition of microorganisms. Further work on the phytoconstituents isolation and purification of the bioactive components are recommended.

Conclusively, the antibacterial activity exhibited by the crude extracts against clinical isolates of *E. coli* (21mm) and *S. aureus* (20mm) that are associated with various infectious diseases, has provided scientific justification in this research for the ethno medicinal uses of the plant in Zaria, Kaduna State, Nigeria.

Acknowledgement

We wish to acknowledge the contribution of Ahmadu Bello University, Zaria, for providing the facilities for conducting the research.

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