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Phytochemical Screening and Antimicrobial Activity of Three Medicinal Plants against Urinary Tract Infection Pathogens

R. U. B. Ebana[1], U. O. Edet[1*], U. M. Ekanemesang[1], C. A. Etok[2], G. M. Ikon[1] and M. K. Noble[1]

[1]Deparment of Microbiology, Faculty of Natural and Applied Sciences, Obong University, Obong Ntak, Etim Ekpo LGA, Akwa Ibom State, Nigeria.
[2]Department of Microbiology, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors RUBE, UOE, UME, GMI and MKN designed the study, wrote the protocol and interpreted the data. Authors RUBE, UOE and MKN anchored the field study, gathered the initial data and performed preliminary data analysis. While authors RUBE, UOE and MKN managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Urinary tract infections are a global health issue. Although antibiotics exist, most people for fear of stigmatization often prefer medicinal treatments. Three plants *Acanthus montanus, Aspilia africana* and *Desmodium velutinum* were investigated for their antimicrobial activity against human urinary tract infection pathogens and also screened for phytochemicals. The phytochemical screening and quantification, characterisation of isolates, antimicrobial screening and minimum inhibitory concentration were all done using standard techniques. Replicate readings were then subjected to analysis of variance. The results of the phytochemical screening and quantification of the plants showed the presence alkaloids (2.40 – 3.12%), glycosides (3.50 – 4.20%), saponins (3.02 -- 6.27%), tannins (0.17 – 0.47%), flavonoids (9.22 – 11.42%), polyphenol (9.72 – 9.90%) and

**Corresponding author: E-mail: uwemedet27@gmail.com;*

reducing compounds (7.40- 9.18 mg %) in studied plants. Analysis of the replicate readings showed significance ($p < 0.05$). The results of the microbial characterization showed that isolates were *Esherichia coli, Staphylococcus species* and *Pseudomonas aeruginosa*. The antimicrobial sensitivity assay showed that the isolates responded differently to the test plants extracts. *Staphylococcus species* and *E. coli* were more sensitive to the test extracts than *P. aeruginosa*. The least inhibition was 11.50 mm while the highest was 14.00 mm. Consistently, all the studied plants showed very good inhibition at a minimum inhibitory concentration of 200 mg. The findings in this study confirms their use in the treatment of urinary tract pathogen and the need for further studies aimed at determining the bioactive compounds in these plants.

Keywords: Acanthus montanus; Aspilia africana; Desmodium velutinum; urinary tract pathogens.

1. INTRODUCTION

Urinary tract infections (UTIs) remains one of the most common infections globally with no less than half of all women having atleast one episode of UTIs during their life time [1,2]. They affect any part of the urinary tract which could be the kidney, ureter, bladder and urethra. The causes of UTIs includes sexual intercourse with infected persons, poor hygiene, holding urine longer than necessary, using diaphragm singly or with spermicides or condoms, underlying kidney stones, diabetes, loss of estrogen and catheter [3]. Most common pathogens remain *Esherichia coli* while others include *Staphylococcus species, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella* and so on [1,3]. With a global estimated 150 million cases of UTIs annually, there are concerns that UTIs are on the increase and one reason for this is the emergence of antibiotics resistance amongst UTIs pathogens [2]. As a result of this, alternative strategies have been sought and used in the treatment of UTIs and amongst them is the use of medicinal plants [1,4].

A study in Bangladesh has shown that people with these infections for fear of social stigmatization prefer to visit traditional medicinal healer even when health facilities may be available or within reach. Furthermore, the study identified a number of medicinal plants that are used to treat a variety of UTIs such as cloudy urine, leucorrhoea, menstrual pains, lower abdominal pains, burning sensation, presence of blood or pus or semen in urine, and difficulties in passing urination and frequent or lack of urination, bleeding of the penis and so on. Interestingly, different tribes had different medicinal plant for their treatment of various UTIs ailments and they include plant (s) in the families of acanthaceae, adiantaceae, amarathaceae, apiaceae, apocynaceae, asterceae, bigoniaceae, convolvulaceae, costaceae, cucurbitaceaee, euphorbiaceae, fabaceae, lauraceae, malvaceae, melastomataceae, menispermaceae, nymphaeaceae, rhamnaceae and rutaceae [4].

The use of medicinal plants is not just the custom of the distant past. It is projected that about 70-90% of the world's population still relies on raw herbs and unrefined extracts [5]. In southern Nigeria, a number of medicinal plants are used by locals to treat urinary tract infection and they include *Acanthus montanus*, *Desmodium velutinum* and *Aspilia africana.*

Aspilia africana is a regular weed that is native to forest zones of Nigeria and other West African countries [6]. The flowers and leaves of this plant have several therapeutic uses such as enhanced wound healing and stoppage of bleeding from cuts. The methanolic extract of the leaves is reported to cure malaria and respiratory ailments [7]. Furthermore, the concoctions of the leaves are used to cure eye problems, ringworm and dysentery [8]. Although they are not popular as food, *A. africana* have been shown to be rich in vitamins such as ascorbic acid, niacin, thiamin and riboflavin, and minerals such as calcium, nitrogen, magnesium, potassium, phosphorus, zinc, nickel, lead and boron. They have also been to shown to be rich in phytochemical such as alkaloids, saponins, flavonoids, tannins and phenols [8].

Acanthus montanus commonly called Bear's breeches is another plant that has a place in folk medicine where it is used to manage body aches and pains, cough and even threatened abortion [9]. Phytochemical evaluation of the plant shows that it is rich in steroids, glycosides, alkaloids and tannins amongst others. The roots extracts have been used in the treatment of urinogenital infection, urethral pains and even endometriosis [10]. Using Swiss Mice model, a study has shown that it has potentials as an herbal solution to spermatogenesis malfunction [11].

Desmodium velutinum is a perennial plant that is erect or semi-erect growing up to about 3 meters in length. Like the former plants, it is widely distributed in the tropics and sub-tropics [12]. Traditionally, the extracts have been used in the treatment of aches, pains, diarrhoea and haematuria when used with hot peppers [12]. Furthermore, it is used as a diuretic, laxative, treatment of cough, and fever amongst others. Studies have shown that it is rich in phytochemicals such as tannins, resins, glycosides, saponins and flavonoids [12,13].

Given the medicinal potentials and the dearth of information on these plants despite their uses in the management of urinary tract health infections, the aim of this study was therefore to examine the phytochemicals and antimicrobial activity of these plants extracts against urinary tract infection pathogens.

2. MATERIALS AND METHODS

2.1 Source and Identification of Plants under Study

Freshly harvested leaves of *Acanthus montanus*, *Aspilia africana* and *Desmodium velutinum* used in the study were purchased from Itam marketing company, Uyo, Akwa Ibom State, south South Nigeria. These were identified locally by Locals of Etim Ekpo community and scientifically by Mr Frank Okpoyoye of University of Calabar Botanical Garden and were then held in the plant collection unit of Obong University with Voucher numbers OU001, OU002 and OU003, respectively.

2.2 Collection of Test Bacteria and Characterization

Urinary tract infection pathogens were collected from the University of Uyo Teaching Hospital from patients presenting with signs and symptoms of UTIs. The isolates were re-identified using standard microbiological procedures and they included *Esherichia coli, Staphylococcus species* and *Pseudomonas aeruginosa.* They were appropriately preserved in slants until required for use [15-17].

2.3 Sample Preparation

The fresh leaves were hand-picked into a clean basin and made into a powder as previously described [18-19]. Briefly, the picked leaves were rinse with sterile distilled water and allowed to air dry by spreading it in a clean stainless tray. After which, the leaves were oven dried using an electric oven maintained at 60℃ for 3 hours. Following drying, the resulting leaves were then pulverized using a mortar and pestle to produce a powder. This was repeated for all the leaves and the powders were then stored in sample bottles until required.

2.4 Preparation of Plants Extracts

This was done as previously described [18-19]. Both ethanolic and aqueous extracts were prepared using 95% ethanol and distilled water, respectively. Briefly, the ethanolic extracts of all the plants were prepared by weighing out and dissolving 50 g of the powdered plants in a sterile beaker containing exactly 1000 ml of the ethanol and distilled water. The mixture was stirred vigorously for a few minutes and allowed to stand for 72 hours wrapped with aluminum foil. After which, it was filtered using a Whatman filter paper and the solvent heated in a water bath to evaporate completely to produce a slurry. These were then repeated for all the plants and aqueous extract. These slurries were then stored in McCartney bottles and kept at 4℃ until needed.

2.5 Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing was carried out on the extracts using the discs diffusion method previously reported [18-20]. Briefly, a cork borer was used to cut filter papers into tiny disks of 5mm in diameter. The disks were then sterilized using an autoclave set at 121℃ for 15 minutes. Colonies of each test isolates were then sub-cultured on nutrient broth and incubated at 37℃ for 6 hours. They were then inoculated on freshly prepared Mueller-Hinton agar plates. The sterilized filter paper disks were soaked in the respective test extracts and then placed on the plates aseptically. The plates were incubated at 37℃ for 24 hours. After incubation, the zones of inhibition were determined in triplicates and on separate days. The mean values were then determined and recorded.

2.6 Minimum Inhibitory Concentration (MIC)

The MIC of the plants extracts were determined using pour plate technique. Different concentration of 20 mg, 100 mg and 200 mg

were prepared by weighing out 0.10 g, 0.50 g and 1.00 g of each of the extracts and dissolving them in 5 ml of water separately. Sterilized 5 mm discs were soaked in the various concentrations for 10 minutes and allowed to air dry. These were then incubated with the pour plates of the test organisms at 37℃ for 24 hours. The plates were then examined for the lowest inhibitory concentration for each of the plant extracts and test organisms.

2.7 Statistical Analysis

The replicate data obtained in this study were managed and analysed using Statistical package for social science (SPSS) version 21. The results were then presented as mean ± sd (standard deviation). Probability values less than 0.05 were considered significant at 95% level of significance.

3. RESULTS

The results of the present study are presented in the tables below. Table 1 gives the plant part used in this study including their scientific, local and family names. Table 2 shows the results of the phytochemical components that were screened for in this study. The result indicates the presence of phytochemicals such as alkaloids, glycosides, saponins, tannins, flavonoids, polyphenols and reducing compounds in all three plants while phlobatannins, anthraquninones and hydroxymethyl anthraquninones were not detected in all studied plants extracts. Polyphenol was the most abundant in all the plant extracts. Table 3 gives the result of the phytochemical quantification and it shows that *A. africana* had the highest amount of reducing compounds, alkaloids and saponins. However, *A. montanus* had the highest amount of polyphenols while *D. velutinum* had the highest amount of glycosides, tannins and flavonoids. Replicate readings of the phytochemical expressed as mean±sd were significant ($p < 0.05$).Table 4 shows the antimicrobial sensitivity results of the aqueous and ethanolic extracts of the studied plants. None of the isolates were resistant to the plant extracts used. The highest zone of inhibition of 14.00 mm was seen with the ethanolic extract of *A. africana* on *Staphylococcus* species. The result of the minimum inhibitory concentration showed that all the plants under study had a consistent MIC of 200 mg.

Table 1. Plant used in the study

S/N	Scientific name	Family name	Local name	Part used
1	*Acanthus montanus*	Acanthaceae	Mbara ekpe	Leaves
2	*Aspilia africana*	Asteraceae	Edeme edong	Leaves
3	*Desmodium velutinum*	Papilinaceae	Isim oyiot	Leaves

Table 2. Phytochemical screening of *D. velutinum, A. montanus* and *A. africana*

Phytochemicals	*D. velutinum*		*A. montanus*		*A. africana*	
	Eth extract	Aq. extract	Eth extract	Aq. extract	Eth extract	Aq. extract
Alkaloids	++	+	++	+	++	++
Glycosides	++	++	++	+	++	+
Saponins	+	++	+	++	++	++
Tannins	++	+	+	+	++	+
Flavonoids	+++	++	+	++	++	++
Reducing Compounds	+	++	+	++	+	++
Polyphenols	++	+++	++	+++	+++	++
Phlobatannins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Hydroxymethyl anthraquinones	-	-	-	-	-	-

+ = positive and - = negative

Table 3. Quantitative estimation of crude phytochemicals

Phytochemicals (%)	*D. velutinum*	*A. montanus*	*A. africana*
Alkaloids	2.40±0.10*	2.51±0.02*	3.12±0.02*
Glycosides	4.20±0.10	3.51±0.01	3.70±0.10
Saponins	3.20±0.10	3.02±0.02	6.27±0.02
Tannins	0.47±0.01	0.17±0.01	0.43±0.02
Flavonoids	11.42±0.02	9.22±0.02	11.32±0.02
Polyphenol	9.72±0.02	9.90±0.02	9.83±0.02
Reducing compounds (mg %)	8.25±0.02	7.40±0.10	9.18±0 .02

**Represent significant Mean±SD that were significant across each column (p < 0.05)*

Table 4. Antimicrobial sensitivity of the extracts (mm)

Isolates	AM		AA		DV	
	Aq	Et	Aq	Et	Aq	Et
E. coli	13.00±0.08	13.00±0.02	11.00±0.01	13.00±0.20	12.00±0 .02	11.00±0.01
P. aeruginosa	11.00±0.40	12.00±0.08	11.00±0.02	12.00±0.01	11.00±0 .02	12.00±0.80
Staphylococcus species	12.00±1.14	12.00±0.70	12.00±1.20	14.00±0.02	12.00±0 .08	12.00±0.20

Key: AM= Acanthus monthanus, AA= Aspilia africana, DV = Desmodium velutinum, Et= ethanol extract and Aq = aqueous extract

Table 5. Minimum inhibitory concentration of the plant extracts

Isolates	AM			AA			DV		
	20	100	200	20	100	200	20	100	200
E. coli	-	-	++	-	±	+++	-	±	++
P. aeruginosa	-	±	+++	-	-	+++	-	±	++
S. aureus	-	±	+++	-	±	+++	-	-	++

Key: AM= Acanthus monthanus, AA= Aspilia africana, DV = Desmodium velutinum, - = no inhibition, ± = sparse, ++ = good and +++ = very good inhibition

4. DISCUSSION

Acanthus montanus, Aspilia africana and *Desmodium velutinum* are otherwise known locally as “Mbare ekpe”, “Edeme edong” and “Isim oyiot” respectively in Akwa Ibom State Nigeria. Although not commonly used as edible vegetables, they have an established place in folk medicine in the treatment of urinary tract infections in south eastern Nigeria. Phytochemical screening of the leaves showed an abundance of phytochemicals. All the leaves are found to contain saponins, glycosides, saponins, flavonoids, tannins, polyphenols and reducing compounds. The most abundant phytochemical was polyphenol that was present atleast in excess in all the leaves extracts. An earlier study showed the presence of tannins, alkaloids, saponins, steroids, hydrogen cyanide, carbohydrate, flavonoids, reducing sugars and terpenoids in *D. velutinum* collected in Enugu, South East Nigeria and this conforms to the findings in our study [21]. Alkaloids, steroids and proteins were absent in the leaves of *D. velutinum* harvested from Anambra State but it was found to have glycosides, flavonoids, tannins, resins and saponin [22].

The phytochemicals recorded in our study for *A. africana* were similar to that found in another study [8]. The presence of alkaloids, saponins, flavonoids, tannins and phenols is similar to the findings in our study. However, on quantification, the most abundant phytochemical was alkaloid in their study while it was flavonoid in our study. *Acanthus montanus* have been shown to be rich in phytochemicals such as alkaloids, flavonoids, phenols, saponins, steroids and tannins [23]. In this study, on quantification, the most abundant phytochemical was steroids followed by flavonoids. This was contrary to our findings which gave flavonoids as the most abundant followed by polyphenol.

The result of the antimicrobial sensitivity of the leaves of *A. montanus*, *A. africana* and *D. velutinum* shows that the extracts have antibacterial activity on urinary tract isolates. The

highest zone of inhibition seen on the isolates was 14.00 mm using ethanolic extract of *A. africana* with *Staphylococcus species*. All extracts whether aqueous or ethanolic showed inhibitory effects on the isolates used in this study. Zones of 27.50 mm, 21.00 mm, 22.50 mm, 25.00 mm, 19.00 mm and 18.50 mm were reported against *E. coli, S. aureus, S. pneumoniae, P. mirabilis, P. aeruginosa* and *K. pneumonia* with *A. montanus*. These zones were far greater than that reported in our study [23]. Our findings are more agreeable to those reported earlier [10]. In their study, they reported zones of 15.00 mm and 19.30 mm for 100 mg/ml of the aqueous extract of *A. montanus* against *P. aeruginosa* and *S. aureus*, respectively. The MIC for all the medicinal plants under study was consistent at 200 mg.

5. CONCLUSION

The present study shows the presence of phytochemicals and the antimicrobial potential of the medicinal plants against urinary tract isolates. The findings in this study confirm their use as medicinal plants against urinary tract infection pathogens and thus the need for further exploitation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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