

Phytochemical screening and antimicrobial activity of three medicinal plants against urinary tract infection pathogens**ABSTRACT**

Urinary tract infections are a global health issue. Although antibiotics exist, most people for fear of stigmatization prefer medicinal treatments. Three plants *Acanthus montanus*, *Aspilia africana* and *Desmodium velutinum* were investigated for their antimicrobial activity against human urinary tract infection pathogens as well as their phytochemical screening. 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 reducing compounds (7.40- 9.18 mg %). 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 11.50mm while the highest was 14.00mm. Consistently, all the studied plants showed very good inhibition at a minimum inhibitory concentration of 200mg. The findings in this study confirms their use in the treatment of urinary tract pathogen and the need for further study aimed at determining the bioactive compounds in these plants.

Keywords: *Acanthus montanus*, *Aspilia africana*, *Desmodium velutinum*, urinary tract pathogens

INTRODUCTION

Urinary tract infections (UTIs) remains one of the most common infection 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 urinary tract infections include 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 *Escherichia coli*. 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, cucurbitaceae, 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 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 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.

MATERIALS AND METHODS

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.

Collection of test bacteria and characterisation

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 *Escherichia coli*, *Staphylococcus species* and *Pseudomonas aeruginosa*. They were appropriately preserved in slants until required for use [15]-[17].

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°C for 3hours. Following drying, the resulting fruits were then pulverized using a mortar and pestle to produce a

109 powder. This was repeated for all the leaves and the powders are then stored in sample
110 bottles until required.

111 **Preparation of plants extracts**

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

121 **Antimicrobial sensitivity testing**

122 Antimicrobial sensitivity testing was carried out on the extracts using the discs diffusion
123 method previously reported [18][19][20]. Briefly, a cork borer was used to cut filter
124 papers into tiny disks of 5mm in diameter. The disks were then sterilized using an
125 autoclave set at 121°C for 15 minutes. Colonies of each test isolates were then sub-
126 cultured on nutrient broth and incubated at 37°C for 6 hours. They were then inoculated
127 on freshly prepared Mueller-Hinton agar plates. The sterilized filter paper disks were
128 soaked in the respective test extracts and then placed on the plates aseptically. The
129 plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition
130 were determined in triplicates and on separate days. The mean values were then
131 determined and recorded.

132 **Minimum Inhibitory Concentration (MIC)**

133 The MIC of the plants extracts were determined using pour plate technique. Different
134 concentration of 20mg, 100mg and 200mg were prepared by weighing out 0.10g, 0.50g
135 and 1.00g of each of the extracts and dissolved in 5ml of water separately. Sterilized 5
136 mm discs were soaked in the various concentrations for 10minutes and allowed to air
137 dry. These were then incubated with the pour plates of the test organisms at 37°C for 24
138 hours. The plates were then examined for the lowest inhibitory concentration for each of
139 the plant extracts and test organisms.

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141 **Statistical analysis**

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

146 RESULTS

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

164 **Table1: Plant used in the study**

S/N	Scientific Name	Family name	Local name	Part used
1	<i>Acanthus montanus</i>	Acanthaceae	Mbara ekpe	Leaves
2	<i>Aspilia africana</i>	Asteraceae	Edeme edong	Leaves
3	<i>Desmodium velutinum</i>	Papilionaceae	Isim oyiot	Leaves

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

	<i>D. velutinum</i>		<i>A. montanus</i>		<i>A. africana</i>	
Phytochemicals	Eth extract	Aq. Extract	Eth extract	Aq. Extract	Eth extract	Aq. Extract

Alkaloids	++	+	++	+	++	++
Glycosides	++	++	++	+	++	+
Saponins	+	++	+	++	++	++
Tannins	++	+	+	+	++	+
Flavonoids	+++	++	+	++	++	++
Reducing Compounds	+	++	+	++	+	++
Polyphenols	++	+++	++	+++	+++	++
Phlobatannins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Hydroxymethyl anthraquinones	-	-	-	-	-	-

169 **+ = positive and - = negative**

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171 **Table 3: Quantitative estimation of crude phytochemicals**

Phytochemicals (%)	<i>D. velutinum</i>	<i>A. montanus</i>	<i>A. africana</i>
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

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

173

174 **Table 4: Antimicrobial sensitivity of the extracts (mm)**

Isolates	AM		AA		DV	
	Aq	Et	Aq	Et	Aq	Et
<i>E. coli</i>	13.00±0.08	13.00±0.02	11.00±0.01	13.00±0.20	12.00±0.02	11.00±0.01
<i>P. aeruginosa</i>	11.00±0.40	12.00±0.08	11.00±0.02	12.00±0.01	11.00±0.02	12.00±0.80
<i>Staphylococcus species</i>	12.00±1.14	12.00±0.70	12.00±1.20	14.00±0.02	12.00±0.08	12.00±0.20

175 **Key:** AM= *Acanthus monthanus*, AA= *Aspilia africana*, DV = *Desmodium velutinum*, Et=

176 ethanol extract and Aq = aqueous extract.

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178 **Table 5: Minimum Inhibitory concentration of the plant extracts**

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

179 **Key:** AM= *Acanthus monthanus*, AA= *Aspilia africana*, DV = *Desmodium*
180 *velutinum*, - = no inhibition, $\pm$ = sparse , ++ = good and +++ = good inhibition.

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182 DISCUSSION

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

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

206 The result of the antimicrobial sensitivity of the leaves of *A. montanus*, *A. africana* and
207 *D. velutinum* shows that the extracts have antibacterial activity on urinary tract isolates.
208 The highest zone of inhibition seen on the isolates was 14.00mm using ethanolic extract
209 of *A. africana* with *Staphylococcus species*. All extracts whether aqueous or ethanolic
210 showed inhibitory effects on the isolates used in this study. Zones of 27.50mm,
211 21.00mm, 22.50mm, 25.00mm, 19.00mm and 18.50mm were reported against *E. coli*, *S.*
212 *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 15.00mm and 19.30mm for 100mg/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 200mg.

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.

COMPETING INTERESTS

Authors have declared that no competing interests.

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