

## Phytochemistry and Antibacterial Activity of *Androgravis Paniculata* (Vinegar Plant) Against Clinical Bacterial Isolates from Diarrheic Patients in Southern Nigeria

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### ABSTRACT

Ethanollic and aqueous extracts of *Androgravis paniculata*, (Vinegar plants) were screened for their phytochemical composition and antibacterial activity against *Shigella dysenteriae*, *Escherichia coli*, *Klebsiela pneumoniae*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Samonella typhi*, *Proteus vulgaris* and *Vibrio* species isolated from diarrhoea stool samples. The phytochemical analysis revealed that *A. paniculata* contained alkaloids, tannins, sterols, saponins and cardiac glycosides. The antibacterial potency determined by paper disc diffusion procedure showed that the extracts exhibited appreciable inhibitory activity on the test isolates. The ethanolic extract showed more therapeutic potentials by inducing inhibitory halo of between  $2.00 \pm 0.3$  and  $16.00 \pm 0.6$  mm in diameter as against  $0.00 - 7.00 \pm 0.5$  mm reported for aqueous extract. The activity index (Xi) for ethanolic extract revealed comparable efficacy with the reference antibiotic (Ampicillin at  $20\text{mgm}^{-1}$ ) against *S. dysenteriae* (1.20) and *S. typhi* (1.38) at  $P=0.05$ . Though the results obtained in this study is promising and a pointer to the possible use of this plant for antibacterial drug against human diarrhoea, detail toxicological investigation and purification of the active compounds is necessary.

**Keywords:** Phytochemical, Antibacteria, *Androgravis paniculata*.

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### INTRODUCTION

Plant's therapeutic essences are secondary metabolites known as phytochemicals. These aromatic chemicals are stored in matured cells of various organs, such as roots, stems, leaves, flowers, fruits and seeds [1]. The synthesized aromatic substances (metabolites) are used by plants as defensive molecules against predation by microorganisms, insects, rodents, herbivores and omnivores [2]. Some of these may be involved in plant odour (terpenoids), pigmentation (tannins and quinines) and flavour (capsacin) [3]. These defensive molecules gives plants their medicinal value which is appreciated by human beings because of their great importance in health care of individuals and communities. Plant derived medicines have made significant contributions to human health and well being as they are usually employed in traditional medicine as remedies for various ailments [4]. Determination of antimicrobial effectiveness and potential of plant extracts against specific pathogens is essential for proper therapy [5]. Systematic screening of plant materials is an important effort to find new bioactive compounds with the needed therapeutic potential to fight against microbial pathogens and understanding the chemical structures of some of these compounds has led to the synthesis and production of more potent and safer drugs [6].

However, the increasing use or overuse of drugs in the treatment of bacterial infections has led to increase in pathogenic organisms that are resistant to available antibiotics. Consequently, it is necessary to increase administered doses, combine antibiotics or provide new antibiotic with lesser tendency for pathogenic organisms to develop resistance to [7]. The rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antimicrobial sources [8, 9].

*Androgravis paniculata* known as Vinegar plant is a mesophytic perennial shrub, 30 – 60cm in height with small elliptic, lanceolate leaves averaging 3cm in width and 8 cm in length. The leaves are dark green in colour with a characteristic odour and intensely bitter. Although vinegar has no recorded origin, they are found wild or cultivated abundantly in the South-South region of Nigeria. The leaves decoction of this plant had been implicated in folk medicine for the treatment of malaria, diarrhoea, body pains and enteritis. Because of its abundant and widespread availability, this plant is studied as part of the exploration for new and novel bioactive compounds against diarrhoeal diseases. This study sets out to determine the phytochemistry and antibacterial activities of *Androgravis paniculata* cultivated in Southern Nigeria.

## **MATERIALS AND METHODS**

### **Preparation of Plant Extracts**

Leaves of *A. paniculata* (Plate 1) were collected in July, 2011 from a garden in Eniong-offot, Uyo located in Southern Nigeria and were authenticated by a taxonomist in the Department of Botany and Ecological studies, University of Uyo. The leaves were sun dried and grounded into fine powder using laboratory plant mill (Christy and Norris Ltd., Chemsford, England) before extraction. The extractive process followed the methods of Harbone [10] and Ogbonna *et al.*, [11]. Precisely 120g of powdered leaf of *A. paniculata* was cold extracted in 250ml of ethanol (96%) (AnalaR-grade) in a soxhlet apparatus for 24hr. Another 120g of plant material was extracted in water for 4 days with occasional shaking. The extracted materials were then filtered through Whatman's No. 1 filter paper. The ethanolic extract was further concentrated using a rotary evaporator to remove the ethanol. The extracts were stored in sterile bottles at 4°C till required.

### **Phytochemical Screening**

Phytochemical properties of *A. paniculata* were determined qualitatively according to the methods of Ramkumar *et al.*, [12] with slight modification.

### **Test for Saponins**

The extract (5ml) was vigorously shaken with 10ml of distilled water for 2 minutes. The formation of persistent foam (froth) for at least 15 minutes indicated the presence of saponins [13].

### **Test for Tannins**

Exactly 0.5g of powdered plant materials was boiled in 20ml of water in a boiling tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for development of brownish green or blue, black colour [14].

### **Test for Alkaloids**

Five grams (5g) of powdered plant sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 48hrs. After filtration, the extracts were concentrated in a water bath to about one quarter of the original volume. Concentrated NH<sub>4</sub>OH was added in drops to the filtrate until an orange precipitate was formed [13].

### **Test for Sterols**

Thin layer of chromatographic plates of the extract were sprayed with Carr-price reagent. A range of colours were produced in both daylight and UV light on heating the sprayed plates for 10 minutes at 100°C. Different colours indicated the different triterpenoids [13].

### **Test for Cardiac Glycosides**

The salkowski test [13] was adopted. Exactly 0.5g of powdered plant samples was dissolved in 2ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown colouration at the interphase indicated the presence of a steroidal ring of cardiac glycoside.

### **Determination of Antibacterial Activity of Crude Plant Extracts**

Antibacterial activity of the aqueous and organic (ethanolic) extracts of plant samples were evaluated by the paper disc diffusion method [14, 15].

### **Test Bacterial Isolates**

Clinical bacterial isolates from diarrhoeal stools of patients attending University of Uyo Teaching Hospital, Uyo, were used for the antibacterial assay. The bacteria were characterized according to the schemes of Cheesbrough [16], as *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumonia*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Salmonella typhi*, *Proteus vulgaris* and *Vibrio* species. The bacteria cultures were maintained on Nutrient Agar at refrigeration temperature prior to testing.

### **Antibacterial Assay**

Precisely 38g of Mueller-Hinton Agar (MHA) medium were dissolved in 1000ml of distilled water, boiled and thereafter sterilized by autoclaving at 121°C for 15 minutes. Under aseptic conditions, in the laminar flow hood, 15mls of MHA was dispensed into pre-sterilized Petri dishes to yield a uniform depth of 5mm. The inocula of the test bacterial isolates were prepared from a 24hr old broth culture. To standardize the inocula, the absorbance was

read at 530nm and adjusted with sterile distilled water to match that of 0.5 McFarland standards. From the prepared bacterial solutions, a final concentration of  $10^6$  colony forming units (cfu) per milliliter of each of the test isolates was prepared. Precisely, 0.2ml each of the bacterial suspension was spread plated on the MHA medium. The plates were allowed to stand for 2 hrs for the test bacterial isolates to be fully embedded and well established in the medium.

The paper disc diffusion technique [17] was adopted. The discs were placed in McCartney bottles and sterilized in an autoclave at  $121^\circ\text{C}$  for 15 min. The bottle was transferred into a hot air oven at  $80^\circ\text{C}$  to dry for 1hr. Ethanolic and aqueous extracts of leaves (10ml each) was transferred into sterile McCartney bottles containing sterile discs. The discs were allowed to soak in extracts for 3 hrs for proper absorption, dried and placed on the seeded MHA agar surface with flamed forceps, and then gently pressed down to ensure contact with the agar surface. Paper discs impregnated with solution containing  $20\text{mgml}^{-1}$  of Ampicillin to serve as positive control and sterile water as negative control were also placed on the MHA medium. The plates were made in triplicates with the discs spaced far enough to avoid overlapping rings of inhibition.

Thereafter, the assay plates were incubated for 24hrs at  $37^\circ\text{C}$  in an inverted position [18]. The sensitivity of the test organisms to the antimicrobial were indicated by clear halo around the disc (zone of inhibition). The diameter of the halo in the triplicate plates was measured by calculating the difference between disc diameter (5mm) and the diameter of inhibition [17]. The mean diameter of inhibition was designated as  $X_m$  and the activity index calculated as the ratio of zone of inhibition of extracts to that of the standard drug. This was designated as  $X_i$  [19].

#### Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The estimation of MIC of the crude extracts was carried out using the methods of Akinpelu [20] and Igbiosa *et al.*, [21]. Two-fold dilution of the crude extracts was prepared and 1ml aliquot of different extract concentrations was added to 9ml of series of pre-sterilized tubes of nutrient broth. Standard suspension of each test organism was inoculated into the tubes and then incubated for 24 hrs at  $37^\circ\text{C}$ . The MICs were read as the least concentration that inhibited the growth of the test organisms. MBCs were determined by selecting tubes that showed no growth during MIC determination. A loopful from each tube was sub-cultured onto extract free medium and incubated for further 24hrs at  $37^\circ\text{C}$ . The least concentration in the MIC test at which no growth occurred was noted as the minimum bactericidal concentration. The data obtained were analyzed using mean, standard deviation and Analysis of variance (ANOVA) [22].

### RESULTS AND DISCUSSION

Results presented in Table-1, showed the qualitative phytochemistry of *A. paniculata* leaves extracted with water and ethanol. The results revealed the presence of saponins, tannins, alkaloids, sterols and cardiac glycosides in both aqueous and ethanolic extracts, though at varying levels. Their presence might be responsible for the remarkable anti-bacterial activity of *A. paniculata*. The results in Table-2, revealed variation in levels of activity induced by the aqueous and ethanolic extracts of *A. paniculata* on the test organisms. Although statistically insignificant ( $P=0.05$ ), the ethanolic leaf extracts exhibited stronger inhibitory effect with diameter inhibition range of  $2.00 \pm 0.3 - 16.00 \pm 0.6\text{mm}$  than  $0.00 - 7.00 \pm 0.5\text{mm}$  recorded for aqueous extract. The results further revealed higher activity index of ethanolic extract against *S. typhi* (1.38), *S. dysenteriae* (1.20), *C. jejuni* (0.80), *Y. enterocolitica* (0.80), and *K. pneumoniae* (0.80).

Diarrhoeal illness is a common bacterial infection of the intestine. In developing countries, one out of five children die of diarrhoea before the age five years [23]. Various causative agents can be responsible for this illness including *C. jejuni* (small Gram-negative comma or S-shaped microaerophilic organism, commonly contracted from fowl), *Salmonella*, *Shigella*, *Proteus*, *Vibrio*, *E. coli*, and *Y. enterocolitica* assayed in this research. The inhibitory effect of the extract of *A. paniculata* against these pathogenic bacterial strains is a pointer to the possible use of the plant as drug for the treatment of diseases caused by these pathogenic isolates. However, the clinical isolates of *Vibro* from diarrhoea stool were apparently very resistant to both ethanolic and aqueous extracts of *A. paniculata*, though sensitive to  $20\text{mgml}^{-1}$  of Ampicillin(positive control) which also inhibited all the test isolates.

The minimum inhibitory concentration (MIC) of the ethanol and aqueous extracts against the test isolates ranged between  $2.50$  and  $7.50\text{mgml}^{-1}$  and from  $10.00$  to  $20.00\text{mgml}^{-1}$  respectively, while the MIC of Ampicillin (positive control) ranged between  $0.25$  and  $5.00\text{mgml}^{-1}$ . On the other hand the minimum bactericidal concentration (MBC) of ethanolic extract ranged between  $5.00$  and  $12.50\text{mgml}^{-1}$  as against  $15.00$  and  $20\text{mgml}^{-1}$  recorded for aqueous extract (Table 3). The higher inhibitory effect of ethanolic extract compared to water extract agrees with the report of Veronika *et al.*, [19] and may be explained by higher proportion of organic soluble constituent in *A. paniculata*. In other words, the inhibitory compounds were extractable more by the ethanolic solvent [2]. Its potency compared

favourably with the highly rated antibiotic (Ampicillin) assayed in this study. The results of the activity index revealed that ethanolic extracts of *A. paniculata* was highly effective against *S.typhi*, *S. dysenteriae*, *C. jejuni*, *Y. enterocolitica* and *K. pneumonia*. The higher MIC values of aqueous extract observed for *S. dysenteriae*, *K. pneumonia*, *Y.enterocolitica* and *C. jejuni* tend to confirm the lower efficacy of the aqueous extract.

Nevertheless, a problem in the use of medicinal plants is the quantity required to effect a cure though this could be overcome if in-depth quantitative analysis is carried out. However, the reduced efficacy of the extracts relative to the reference drug may be due to the fact that, they are still crude and require further purification [2].

**CONCLUSION**

Leaves of *Androgravis paniculata* contain medicinally useful aromatic chemicals such as sterols, tannins, cardiac glycoside and alkaloids, which are responsible for the antibacterial activities of the extracts especially the ethanolic extract against bacteria species isolated from diarrhoea stools. The observed effects on the test organisms, though *in vitro*, appear interesting and promising and therefore presents an opportunity for the use of this plant in antibacterial drug development for human diarrhoea. However, detail effect of this extract on the test organisms, more toxicological investigations and further purification of the bioactive ingredients need to be carried out.

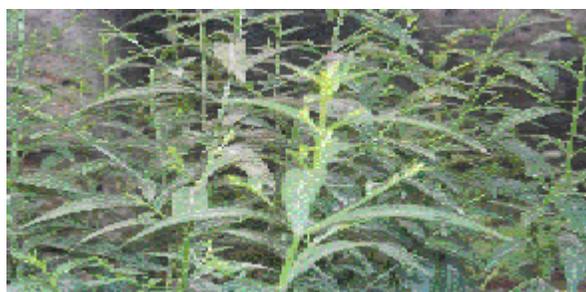


Fig.-1: Vinegar Plant (*Androgravis paniculata*)

Table-1: Phytochemical Properties of Ethanolic and Aqueous Extracts of *Androgravis paniculata*

Test	Ethanol	Water
Alkaloid	+++	+
Tannin	+	-
Sterol	++	+
Saponin	+++	+
Cardiac glycoside	+++	+

+ = Present in small amount, ++ = moderately present, +++ = present in large amount

Table-2: Antibacterial Activities (mm of halo diameter) Profile of Ethanolic and Aqueous Extracts of *Androgravis paniculata*

Isolates	Ethanolic Extract		Aqueous Extract		Antibiotic (Ampicillin)
	Xm	Xi	Xm	Xi	Xm
<i>S. dysenteriae</i>	12.0 ± 0.0	1.20	4.0 ± 0.2	0.40	10.0 ± 0.2
<i>E. coli</i>	11.0 ± 0.2	0.73	7.0 ± 0.5	0.46	15.0 ± 0.1
<i>K. pneumonia</i>	8.0 ± 0.3	0.80	0.0 ± 0.0	0.00	10.0 ± 0.3
<i>Y. enterocolitica</i>	16.0 ± 0.6	0.80	5.0 ± 0.4	0.25	20.0 ± 0.2
<i>C. jejuni</i>	15.0 ± 0.5	0.83	5.0 ± 0.2	0.27	18.0 ± 0.3
<i>S. typhi</i>	11.0 ± 0.4	1.38	4.0 ± 0.3	0.50	8 ± 0.1
<i>P. vulgaris</i>	14.0 ± 0.2	0.93	7.0 ± 0.1	0.46	15.0 ± 0.4
<i>Vibrio sp.</i>	2.0 ± 0.3	0.50	0.0 ± 0.0	0.00	10 ± 0.10

Xm = Mean value of triplicate zone of inhibition

Xi = Activity index

Table-3: The MIC and MBC regimes of Ethanolic and Aqueous Extracts of *A. paniculata*

Test bacteria	Ethanol Extracts (20mgml <sup>-1</sup> )		Aqueous Extracts (20mgml <sup>-1</sup> )		Antibiotic (Ampicillin) (20mgml <sup>-1</sup> )
	MIC	MBC	MIC	MBC	
<i>S. dysenteriae</i>	5.0	10.0	18.0	NA	2.50
<i>E. coli</i>	7.5	15.0	15.0	18.0	5.00
<i>K. pneumonia</i>	5.0	10.0	18.0	15.0	2.50
<i>Y. enterocolitica</i>	2.5	5.0	10.0	16.0	0.25
<i>C. jejenum</i>	5.0	7.5	18.0	16.0	0.50
<i>S. typhi</i>	5.0	12.5	NA	NA	2.00
<i>P. vulgaris</i>	2.5	5.0	10.0	15.0	0.25
<i>Vibrio</i> sp.	0.00	0.00	0.00	0.00	2.00

NA = Not active at (20mgml<sup>-1</sup>), the highest concentration tested

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