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Evaluation of Antibacterial Properties of Stem Extract of *Andrographis paniculata*- Nees on Some Antibiotic Resistant Bacterial Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Author EOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AOO and TTA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this research is to investigate the antibacterial and phytochemical properties of stem extract of *Andrographis paniculata* (Nees). The phytochemical and antibacterial activities of the stem of *Andrographis paniculata* were determined using standard methods. The phytochemical analysis revealed the presence of saponin, tannin, terpenoid, phlobotannin and flavonoid in methanol, n-hexane, and aqueous extracts, but there was absence of tannin in the aqueous extract. Saponin was the most abundant in the methanol extract with the value of 20.22 mg/ml, followed by the n-hexane extract (19.56 mg/ml) while the least was recorded against the aqueous extract (15.34 mg/ml). Flavonoid and terpenoid were present in appreciable amount in all the extracts. Resistant pattern against the antibiotics considered are as follows: Gentamycin (56%), Cefuroxime (100%), Ceftazidime (100%), Augmentin (100%), Ofloxacin (33%), Ampicillin (100%), Nitrofurantoin (67%), Ciprofloxacin (50%), Ceftriaxione (100%), Erythromycin (33%) and Cloxacin (100%). *S. typhi, P. aeruginosa, S. dysentriae,* and *P. mirabilis* showed complete resistance to all the antibiotics used

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while on the gram positive bacterial isolates, *B. cereus* was resistant to all the antibiotics used. All the bacterial isolates were susceptible to n- hexane and methanol extracts with the zone of inhibition ranging from 10-17 mm and 11-28 mm respectively. The aqueous extract did not exert much antibacterial activity on the tested isolates. *Pseudomonas aeruginosa* isolated from wound was the most susceptible to the methanol extract with the value of 28 mm. All the gram positive isolates used were more susceptible to the n- hexane extract such as *S. aureus, E. faecalis* and *B. cereus* with the zone of inhibition of 17 mm, 19 mm and 16 mm respectively. The Minimum Inhibitory Concentrations ranged between 3.125 mg/ml and 50 mg/ml. Findings from this study suggest that the stem extract of *A. paniculata* especially methanolic extract possess broad spectrum activities against the antibiotic resistant isolates tested, hence could be used for the treatment of infections implicated by the bacterial used in this study.

Keywords: Andrographis paniculata; minimum inhibitory concentration; phytochemical.

1. INTRODUCTION

Bacterial resistance to the antibiotics currently in use poses major threat to public health which has deleterious effects on human medicine worldwide. The emergence of multi-drug resistant organisms in the community, especially hospital environment is alarming and the infections caused by these organisms are associated with increased mortality [1,2]. In the USA, about 23,000 people die yearly due to infection caused by antibiotic-resistant organism [3]. According to a recent report by the Centers for Disease Control and Prevention which states by 2050 there would be about 300 million premature death with a loss of up to $100 trillion (£64 trillion) to the global economy [3]. Alternative therapy is on the search as a result of the increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents and this has led to the screening of several medicinal plants for their potential antimicrobial activity [4] WHO [5] has estimated conservatively that, between 60 and 90 percent of the population of the non-industrialized countries rely on medicinal plants to meet their health care needs, either totally or partially. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. According to Ahmed and Urooj [6], these phytochemical substances are non-nutritive substances, and have protective or disease preventive property. Infections caused by antibiotic resistant pathogens are on the increase and this has been reported to have deleterious effects on human and economy globally due to the failure of antibiotics and its associated side effects. Hence, the search for alternative therapy.

Andrographis paniculata, commonly known as 'King of Bitter is a small, annual, branched and erect plant belongs to the family Acanthaceae. In Yoruba language, it is called Awere. It is an annual, branched, erect- running ½ to 1 meter in height. The leaves are 7.5 cm Long and 2.5 cm wide. The flowers of plant is of white colour .The seeds are small in size of yellowish brown n colour. In ethnomedicine, the leaf and the root of *Andrographis paniculata* has been reported in the treatment of array of diseases such as cancer, diabetes, high blood pressure, snake bites, skin diseases etc Sivananthan and Elamaran, 2013. Hence, this work made an attempt to study the antibacterial activity of the stem extract of *Andrographis paniculata* against various antibiotic resistant pathogenic organisms.

2. MATERIALS

2.1 Sample Collection, Identification and Drying

Andrographis paniculata (Awere) was collected from shagari village in Akure, Ondo State and authenticated at the herbarium section of the Department of Botany, Obafemi Awolowo University, Osun State, Nigeria. The stem back of *Andrographis paniculata* was separated from the leaf and dried for about three weeks and ground with a blender to obtain a fine powder after which it was stored in an air tight container prior to use as previously described by Nweze, et al. [7].

2.2 Methods

2.2.1 Test organisms

The clinical bacterial isolates were collected from FMC, Owo, Ondo State. The bacterial isolates

were identified and maintained on agar slants prior to use.

2.2.2 Extraction of plant sample

The extraction was performed by adding a 150 g each of the sample in 1000 ml of each solvent in a sterile container, swirled to ensure effective missing and stoppered to avoid loss of volatile liquid at ambient temperature (28±2ºC) for 48 h. The mixture was extracted by agitation on a rotary shaker. After 48h, the mixture was decanted using muslin cloth followed by Whatmann NO 1 filter paper. The filtrates were then allowed to evaporate to dryness at room temperature. The concentrated extract was scooped into a pre-weighed small sterile container and weigh. The percentage yield was calculated as recovered weight of extract divided by the amount of powdered sample soaked in solvent multiply by 100. The container was covered, labeled accordingly and stored in the refrigerator and kept for prior use as previously described by Nweze, et al. [7].

2.2.3 Phytochemical analysis

The qualitative and quantitative phytochemical analysis of the extracts was determined to reveal the presence of the bioactive components such as; tannins, saponin, terpenoid, Phlobatannin flavonoid, Keller Killani's test, Salkowski's and Lieberman's test. This was carried out by the method of Sofova, et al. [8].

2.2.3.1 Saponin determination

The ability of Saponin to produce frothing in aqueous solution was used as screening test for Saponin. About 0.5 g of extract was shaken with distilled water in a test tube frothing which persist on warming was taken as preliminary evidence for the presence of Saponin.

2.2.3.2 Tannin determination

About 0.5 g of the extract was stirred with 100 ml of distilled water, filtered and ferric chloride reagent was added to the filtrate a blue black green or blue green precipitate was taken as evidence for presence of tannin.

2.2.3.3 Phlobatannin detemination

Deposition of red precipitate when 0.5 g of the extract was boiled with 1% aqueous HCl was taken as evidence for the presence of phlobatannin.

2.2.3.4 Keller- Killiani's test

0.5 g of the extract was dissolve in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layer with 1 ml of conc. H_2SO_4 a brown obtain at the interface indicate the presence of a deoxy sugar. A violet ring may appear below the brown ring while in the acetic acid layer; a green ring may form just above the brown ring and gradually spread throughout this layer.

2.2.3.5 Lieberman's test

20 ml of acetic anhydride was added to 0.5 g of the extract and filter, 2 ml of conc. H_2SO_4 was added to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroids nucleous. (i.e aglycone portion of the cardiac glycosides).

2.2.3.6 Salkowski's test

0.5 g of the extract was mixed with 20 ml of chloroform and filtered 3 ml of conc. H_2SO_4 was added to the filtrate to form a layer. A reddish brown color at the interface was observed which indicate the presence of steroidal ring.

2.2.4 Standardization of inoculum

Freshly prepared nutrient broth was inoculated with test organisms and incubated for 24 h at 37ºC. Exactly 0.2 ml from the cultured broth was aseptically dispensed into 20 ml of freshly prepared nutrient broth and incubated for 3 h at 37ºC to standardized to 0.5 McFarland standard of Barium sulfate solution which is equivalent to 1×10^6CFU [9].

2.2.5 Antibiotic sensitivity test

Antibiotics sensitivity test was carried out on the test bacteria using the method described by Doughari, et al. [10]. A multi-sensitivity disc bearing antibiotics AMP – ampicillin (10 µg), GEN – gentamycin (10 µg), NIT- nitrofurantoin (200 µg), OFL- ofloxacin (5 µg), CPR- ciprofloxacin (5 µg), CRX- cefuroxime (30 µg), CAZ- ceftazidime (30 µg), CTR- ceftriaxione (30 µg), ERY- erythromycin (5 µg), CXC- cloxacin (10 µg), AUG- augmentin (30 µg), was used against each of the test bacteria inoculated on Mueller Hinton agar plates. They were incubated at 37°C for 24 h. After incubation, the diameter of the zone of inhibition around each well was measured to the nearest millimeter. The

experiment was carried out in triplicates and the mean was calculated.

2.2.6 Antimicrobial assay of the extracts

One gram of the extract was measured into a sterile test tube and 10 ml of 20% dimethyl sulfur oxide (DMSO) was added to dissolve it. This gives a 100 mg/ml concentration of the extract and was diluted in two- folds to obtain four different dilutions of the extract such as 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml in addition to the 100 mg/ml concentration. Using a sterile cork borer of diameter 8 mm, 3 wells was dogged into the prepared Mueller Hinton agar medium which was initially flooded with 1 ml of the standardized inoculum of each test organism. Each well was then filled with about 100 µl of the different concentrations of the crude extracts. The plates was left for 1 h at room temperature for diffusion to take place and later incubated for 18-24 h at 37ºC. The experiment was performed in triplicates and the inhibition zone diameter was recorded at the average of the three replicates. This was carried out by the method of Olutiola, et al. [11].

2.2.7 Minimum Inhibitory Concentration of the extracts

Based on the preliminary screening, the extracts that showed potent antimicrobial activity was further tested to determine the minimum inhibitory concentration (MIC) for each bacterial sample. This was done according to the method of Doughari, et al. [10].

2.2.8 Statistical analysis of data obtained

Data obtained were analyzed by two ways analysis of variance (ANOVA) while the means were compared by Duncan's multiple range tests at 95% confidence level using SPSS 16.0 version. Differences were considered significant at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the percentage yield of *Andrographis paniculata* with different solvents. The highest percentage yield was recorded against the methanol extract (11.0%) followed by n-hexane extract (6.8%) while water extract (4.04%) had the least percentage yield. This result agrees with the previous study of Ibrahim, et al. [12] that alcohol especially methanol is a better solvent for more consistent extraction of active substances against microbes from medicinal plant in comparism to other solvents. The presence of the phytoconstituents revealed its medicinal and physiological activities. These constituents have been reported to play vital roles in exerting inhibitory effects on microorganisms Ibrahim, et al. [12]. The qualitative and quantitative phytochemical screening is shown in Table 2 and Fig. 1. The result reveals the presence of medicinally active constituents like tannins, terpenoid, saponin, phlobatanin and flavonoid. There was presence of tannin, saponin and terpenoid in the methanol and n- hexane extracts, only tannin was absent in the aqueous extract. All this phytoconstituents were present in appreciable amounts in the extract. Saponin was found to have the highest value in methanol extract (20.22 mg/ml) followed by the n- hexane extract (19.56 mg/ml). Methanol extract also had the highest value for terpenoid (18.1 mg/ml) followed by the n-hexane extract (17.34 mg/ml). The presence of flavonoids confirm the traditional use of *Andrographis paniculata* as antimalarial agent and their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [13]. Likewise, the presence of tannins and saponins also confirm their use in folkoric medicine as antibacterial agent which inhibit some redox pathways and other biochemical processes in the bacterial cell [14]. Table 3 revealed the antibiotic sensitivity pattern of the bacterial isolates which includes: gentamicin (56%), cefuroxime (100%), ceftazidime (100%), augmentin (100%), ofloxacin (33%), ampicillin (100%), nitrofurantoin (67%), ciprofloxacin (50%), ceftriaxione (100%), erythromycin (33%) and cloxacin (100%). *E. coli* and *P. aeruginosa* was resistant to all the antibiotics except gentamicin, *K. pneumonia* was sensitive to gentamicin, ofloxacin, nitrofurantoin and ciprofloxacin, *S. typhi* was completely resistant to all the antibiotics while on the gram positive isolates, *S. aureus* was resistant to all the antibiotics except ofloxacin whereas *B. cereus* showed complete resistance to all the antibiotics used. Existence of high drug resistance to multiple antibiotics in *E. coli, S. aureus, K. pneumonia, and P. aeruginosa* isolates from the various samples could be attributed to negligence on patients' part, antibiotics misuse, self-prescription, incomplete treatment schedules, misprecription, purchase of over the counter drugs, limited knowledge about multidrug- resistant isolates and antimicrobial resistance among patients [15]. Table 4 shows the antibacterial effect of the extract on some antibiotic resistant isolates. All the isolates were susceptible to n- hexane and methanol extracts

with the zone of inhibition ranging from 13-20 mm and 15-28 mm respectively. *P. aeruginosa* was the most susceptible to methanol extract with the zone of inhibition of 28 mm followed by *S. typhi* with the value of 26 mm. *Pseudomonas aeruginosa* and *S. typhi* have been reported to be implicated in many infections and have been documented to be resistant to many antibiotics. However, the highest zone of inhibition on n-hexane extract was recorded against *E. faecalis* (19 mm) followed by *S. aureus* (17 mm) while aqueous extract did not exert much antibacterial activity.

Table 1. Percentage yield of stem extract of *Andrographis paniculata*

Extracts	Gram Soaked (g)	Gram Extracted (g)	Percentage yield (%)
APNH	150	10.27	6.8
APM	150	16.59	11.0
APA	150	6.06	4.04

The result of the antibacterial activity of the extracts revealed the potentiality of the extracts as the source of new antibacterial agents even against some antibiotic resistant strains. It was observed that the antibacterial activity varied among methanolic, n- hexane and cold water of the extracts used. The result revealed that all the extracts except water have antibacterial activity. Methanol extracts had the highest antibacterial activity against all the tested bacteria isolates. It has been recorded that methanol possess highest solubility/extractability for various plant metabolites, it is found to be more effective against *Pseudomonas aeruginosa* isolated from wound of diabetic patient, and this bacteria has been reported to be resistance to many antibiotics [16]. This study is in accordance with the previous study that methanol fruit extract of *A. paniculata* is potent against gram positive and gram negative organisms (Sharma, et al. 2011). Table 5 revealed the Minimum Inhibitory Concentration which ranged between 3.12-50 mg/ml. The lowest MIC exhibited by methanol extract was recorded against *S. typhi*, *P. aeruginosa* and *S. aureus*. The low MICs (3.13-25.00 mg/ml) observed during this study indicate that the extracts are efficacious and can be used for the management of diseases caused by the test microorganism; hence indicate the therapeutic potential of the extract.

Table 2. Qualitative phytochemical composition of the stem extract of *A. paniculata*

Phytochemicals	APNH	APM	APA
Saponins	+	+	+
Tannins	+	+	-
Phlobotannins	+	+	+
Terpenoid	+	+	+
Killer-Killani's Test	+	+	-
Lieberman's Test	-	-	-
Flavonoid	+	+	+
Salkowski's test	+	-	-

Key: APNH- N-hexane extract of Andrographis paniculata, APM- Methanol extract of Andrographis paniculata, APA- Aqueous extract of Andrographis paniculata, += Presence of Phytoconstituents, - = Absence of Phytoconstituents

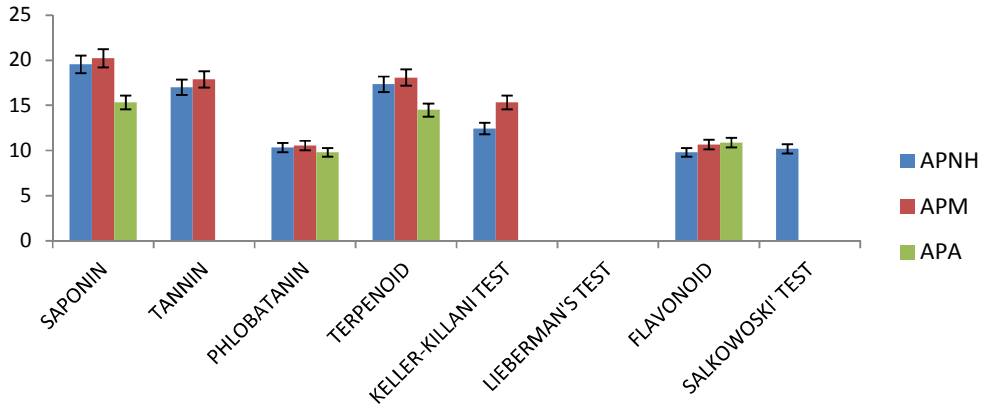

Fig. 1. Quantitative phytochemical composition of the stem extract of *A. paniculata*
Key: APNH- N-hexane extract of Andrographis paniculata, APM- Methanol extract of Andrographis paniculata, APA- Aqueous extract of Andrographis paniculata

Table 3. Antibiotics sensitivity pattern of the bacteria isolates

Test bacteria	GEN	CRX	CAZ	AUG	OFL	AMP	NIT	CPR	CTR	ERY	CXC
E. coli	S	R	R	R	R	R	R	R	ND	ND	ND
K. pneumoniae	S	R	R	R	S	R	S	S	ND	ND	ND
*S. typh*i	R	R	R	R	R	R	R	R	ND	ND	ND
P. mirabilis	S	R	R	R	R	R	R	R	ND	ND	ND
S. dysentriae	R	R	R	R	S	R	R	S	ND	ND	ND
P. aeruginosa	R	R	R	R	S	R	S	S	ND	ND	ND
S. aureus	R	R	R	R	S	ND	ND	ND	R	R	R
E. feacalis	S	R	R	R	S	ND	ND	ND	R	S	R
B. cereus	R	R	R	R	R	ND	ND	ND	R	R	R

Keys: AMP – ampicillin (10 µg), GEN – gentamycin (10 µg), AUG- augmentin (30 µg), NIT- nitrofurantoin (200µg), OFL- ofloxacin (5 µg), CPR- ciprofloxacin (5 µg), CRX- cefuroxime (30 µg), CAZ- ceftazidime (30 µg), CTR- ceftriaxione (30 µg), ERY- erythromycin (5 µg), CXC-cloxacin (10 µg), S-Sensitive, R-Resistant, ND- Not determined

Table 4. Antibacterial activity of the stem extracts of *Andrographis paniculata* (50 mg/ml) on the antibiotic resistant bacteria isolates

Isolates	NHAP	MAP	AQAP
E. coli	10.00 ± 1.15^{a}	16.00 ± 0.58^{b}	8.00 ± 1.15^{c}
K. pneumoniae	$14.00\pm.58^{c}$	12.00 ± 1.15^{a}	6.00 ± 0.00^{a}
S. typhi	15.00 ± 1.15^{d}	26.00 ± 0.58^{g}	0.00 ± 0.00^{a}
P. aeruginosa	14.00 ± 0.58^{c}	28.00 ± 0.58^{h}	0.00 ± 0.00^{a}
P. mirabilis	18.00 ± 0.58^{f}	18.00 ± 0.58^{d}	0.00 ± 0.00^{a}
S. dysentriae	15.00 ± 1.15^{de}	21.00 ± 0.58^{f}	0.00 ± 0.00^{a}
S. aureus	17.00 ± 0.58^{e}	17.00 ± 0.58^{c}	12.00 ± 0.00^{a}
E. faecalis	19.00 ± 0.58^{g}	20.00 ± 0.58^{e}	11.00 ± 0.58^{b}
B. cereus	16.00 ± 0.58^{e}	18.00 ± 0.58^{d}	0.00 ± 0.00^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05)

Key: NHAP- N-hexane extract of Andrographis paniculata, MAP- Methanol extract of Andrographis paniculata, AQAP- Aqueous extract of Andrographis paniculata

Table 5. Minimum Inhibitory Concentration (MIC- mg/ml) of the stem extract of *Andrographis paniculata* (mm) on the antibiotic resistant bacterial isolates

Isolates	NHAP	MAP	AQAP
E. coli	12.5	12.5	50
K. pneumonia	25	25	50
S. typhi	50	3.125	ND
S. dysentriae	25	12.5	ND
P. aeruginosa	25	3.125	ND
P. mirabilis	50	12.5	ND
S. aureus	12.5	3.125	50
E. faecalis	6.25	25	50
B. cereus	6.25	25	ND

Key: NHAP- N-hexane extract of Andrographis paniculata, MAP- Methanol extract of Andrographis paniculata, AQAP- Aqueous extract of Andrographis paniculata

4. CONCLUSION

The stem extract of *Andrographis paniculata* possesses broad spectrum activity against the entire bacterial isolates used to which most bacterial isolates were found resistant to many antibiotics currently in use. The probable bioactive components present in the extract

might be attributed to the inhibitory ability of the extract on the bacterial isolates used. Hence, *Andrographis paniculata* could be used as alternative therapy for the treatment of bacterial infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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