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Phytochemical And Antibacterial Screening Of Andrographis Paniculata (king Of Bitterness) Roots Extracts On Bacteria Isolated From The Foot Ulcers Of Diabetic Patients

Walter, Binyelu Walter and Iloputaife, Jalumchimike

Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Enugu State, Nigeria.

***Author for Correspondence:** walterbinyelu@gmail.com

ABSTRACT

Foot ulcers are common among diabetic patients and can lead to severe bacterial infection. The aim of the study was to determine the antibacterial activity of Andrographis paniculata root extracts on clinical pathogens isolated from foot ulcers of diabetic patients. A total of 5 typed strains comprising of Acinetobacter johnsonii strain JUQ303 Pseudomonas rhodesiae strain YHBT5, Alcaligenes faecalis strain 2, Alcaligenes faecalis strain N148 and Alcaligenes faecalis strain 3 were isolated from patients attending clinics at the National Orthopedic Hospital, Enugu. Roots of Andrographis paniculata were obtained from a local garden in Anaocha local government area of Anambra State. They were pulverized and extracted using ethanol, acetone and water solvents. These crude extracts were reconstituted using 5ml of Dimethyl sulfoxide (DMSO) to obtain concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and1.56mg/ml. The isolates were screened for sensitivity to the plant extracts using agar well diffusion method. Antibiotic susceptibility pattern of these isolates was analyzed using Kirby-Bauer disc diffusion method. The aqueous extract showed highest zone of inhibition of 12mm on Acinetobacter johnsonii, 11mm on Psedomonasrhodesiae, 10mm on Alcaigenes faecalis strain 2, 9mm on Alcaligenes faecalis strain N148 and 7mm on Alcaligenes faecalis strain 3, respectively at the concentration of 200mg/ml. At lower concentrations of 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml, the aqueous extracts showed no inhibition on tested isolates. The MIC of the ethanol root extract of Andrographis paniculata were recorded at 12.5mg/ml,25mg/ml, 50mg/ml, 50mg/ml and 12.5mg/ml on Acinetobacter johnsonii strain JUQ303, Pseudomonas rhodesiae strain YHBT5, Alcaligenes faecalis strain 2, Alcaligenes faecalis strain N148 and Alcaligenes faecalis strain 3, respectively. The MIC of the acetone root extract were recorded at 25mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 100mg/ml on Acinetobacter johnsonii, Psedomonasrhodesiae, Alcaligenes faecalis strain 2,Alcaligenesfaecalis strain, Alcaligenes faecalis strain 3 respectively while the MIC of the aqueous root extract were recorded at100mg/ml, 200mg/ml, 200mg/ml, 100mg/ml and 200mg/ml Acinetobacter johnsonii, Psedomonasrhodesiae, Alcaligenes faecalis strain 2, Alcaligenes faecalis strain, Alcaligenes faecalis strain 3 respectively. The aqueous extract showed no bactericidal effect on tested isolates. From the study, ethanol extract of A. paniculataroots showed the highest antibacterial potency on clinical pathogens isolated from diabetic foot ulcers than the acetone and aqueous extracts. Andrographis paniculata plant is easily accessible, potent, economical and safe to man, therefore, this study encourages the use of plant extracts in the treatment of human diseases caused by these pathogens.

Keywords: Foot ulcers, diabetes, Andrographis paniculata, Acinetobacter johnsonii, Alcaligenes faecalis.

INTRODUCTION

Plants produce a wide range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs used today are acquired from natural resources or semi-synthetic derivatives of natural products used in the traditional systems of medicine. (Kadhim et al. 2020).

Medicinal plants are finding their way into pharmaceuticals, cosmetics and nutraceuticals. In pharmaceutical field, medicinal plants are largely used for broad range of substances present in plants which have been used to treat infectious as well as chronic diseases (Abutbul et al. 2018). The drugs already in use to treat infectious diseases are of concern because drug safety remains a huge global issue. Almost all of the synthetic drugs have side effects; also, most of the microbes develop resistance against drugs. To alleviate this problem, antimicrobial compounds from potential plants should be explored. These drugs from plants have

fewer side effects, less toxic, scanty and also cost effective. They are effective in the treatment of infectious diseases while simultaneously mitigating many side effects that are often associated with synthetic antimicrobials (Okigbo et al. 2019).

Treatment with medicinal plants having antibacterial activity is potentially beneficial alternative and promising source of pharmaceutical agents (Sridevi et al. 2016). Plants are rich in a wide variety of secondary metabolites of phytochemical constituents such as tannins, alkaloids and flavonoids, which act against different diseases (Stefanello et al. 2018). In addition, plant-derived medicines provide a cheaper source of treatment and significant accuracy than chemotherapeutic agents (Tajikarimi et al. 2017).

Andrographis paniculata, commonly known as "king of bitter", is a small, annual, branched and erect plant belonging to the family Acanthaceae. It grows abundantly in Southeastern Asia, including India, Sri Lanka, Java, Pakistan, Indonesia and Malaysia (Hosamani et al. 2017). It is rich in a wide variety of phytochemical constituents such as diterpens, flavonoids and lactones (Kadhim et al. 2020), and a powerful herb, used to control fever, sore throat, hepatitis and variety of other chronic and infectious diseases. (Govind and Madhuri, 2018). The herb and its isolates are reported to possess anti-inflammatory activity, hepatoprotective, anti-diabetic, anti-malarial, and antimicrobial activities (Dhawan, 2018). It has also been reported to possess immuno-stimulatory ability when prepared as tonics (Sukanya et al. 2019).

Diabetes Mellitus is a metabolic endocrine disorder due to overall deficiency of insulin (type 1 diabetes) or defective insulin function (type 2 diabetes) which causes hyperglycaemia (Tepe et al. 2018).

Diabetic foot ulcers are among the most common complications of diabetic patients, which is not well controlled (Yu et al. 2018). These ulcers are usually in areas of the foot, which encounter repetitive trauma and pressure sensation resulted by microbial infections (Wang et al. 2017). About 25% of patients with diabetes mellitus develop foot ulcers and 5% end up with an amputation, which is as a result of progressive infection caused by pathogenic bacteria that are resistant to therapeutic drug treatment regimens (Zaiden et al.2018).

MATERIALS AND METHODS

Collection and Processing of Plant Material

The roots of the plant *Andrographispaniculata* were collected locally from gardens in Aguluzigbo Anaocha Local Government Area of Anambra State. It was identified by a taxonomist in the Department of Applied Biology and Biotechnology of the Enugu State University of Science and Technology (ESUT). The roots were washed thoroughly with distilled water and dried for 7 days at a room temperature. Dried roots were blended to powder with the aid of a sterile blender and were stored in an air tight container until required for the analysis.

Preparation of Plant Extracts

A modified method of Abdulrahman et al. (2014) was used. Fifty (50)grams of the ground roots of *Andrographispaniculata* were weighed into three conical flasks containing 200mls of solvent extracts (Aqueous, Acetone and Ethanol) respectively. The conical flasks were covered tightly and left for 48 hours to extract at a room temperature with intermittent shaking. The extracts were filtered aseptically into sterile conical flasks using what-man no 1 filter paper. The ethanol, acetone and aqueous extracts were evaporated using a soxhlet apparatus and the crude extracts obtained were stored at 40C in a refrigerator until used for experimentation.

Standardization of Inoculumn

The test organisms used were *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Alcaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Alcaligenes faecalis* strain 3 that were isolated from foot ulcers of diabetic patients attending clinics at Federal Orthopaedic Hospital Enugu, Enugu State. Confirmatory tests were carried out on each of the organisms using molecular identification and biochemical characterization tests.

McFarland equivalent turbidity standard was prepared. The 0.5 McFarland turbidity standard was used to adjust the turbidity of the inoculum that was used for antimicrobial susceptibility test.

Antimicrobial Susceptibility test using the extracts.

Agar well diffusion method was used to determine the antibacterial activity of the extracts. To test for this, 1g of each of the extracts were

dissolved in 5mls of DMSO respectively and then varying concentrations of the extracts (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml) were obtained.

A standard inoculum of 1.5 x 108 cells *Acinetobacterjohnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Alcaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Alcaligenes faecalis* strain 3 which is equivalent to 0.5 McFarland standards were spread on the surface of sterile Mueller Hinton agar plates in duplicates. A sterile 6mm cork borer was used to make holes on the Mueller Hinton agar plates in which 0.1ml of various concentrations of the extracts were added. The plates were then incubated at 37^0C for 24hours and the zones of inhibition were measured.

Determination of the Minimum Inhibitory Concentrations (MIC)

This was determined using agar well dilution method. 0.1ml of the inoculum (*Acinetobacterjohnsonii* strain JUQ303, *Pseudomonas rhodesiaestrain* YHBT5, *Alcaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Alcaligene faecalis* strain 3 was spread on petri dishes containing Mueller Hinton agar. Wells (6mm diameter) were punched into the already inoculated Mueller Hinton agar plates using sterile cork borer and 0.1ml of each of the extracts (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, and 1.562mg/ml) were added into each of the wells respectively and they were incubated at 37^0C for 24 hours. After the incubation, the MIC was determined as the least concentration of the extract that inhibited the growth of the organism.

Determination of Minimum Bacterial Concentration (MBC)

In this technique, test tubes containing the various concentrations of the extracts were inoculated with 0.1ml of the standardized organisms respectively and were incubated at 37^0C for 24hours.

Test tubes with no visible growth were streaked on various plates containing Mueller Hinton agar and incubated at 37^0C for 24hours. They were observed for the presence or absence of any visible growth. The MBC was taken as the concentration of the plant extract that did not exhibit any bacterial growth after the incubation.

Phytochemical screening of the plant extract

The root extracts were screened for their phytochemical activity (qualitative and quantitative analysis) using standard method.

RESULTS

Table 1: The result of the isolates, strains and accession numbers

Isolates	Strain	Accession number
Acinetobacterjohnsonii	JUQ303	MN826149.1
Pseudomonas rhodesiae	YHBT5	MG571711.1
Acaligenesfaecalis	2	MN636316.1
Acaligenesfaecalis	N148	JQ900529.1
Acaligenesfaecalis	3	MN636317.1

Table 2: Zones of Inhibition of root extracts of Andrographis paniculata on *Acinetobacterjohnsonii* strain JUQ303 MN826149.1

Concentrations (mg/ml)	Zones of Inhibition (mm)		
	Ethanol	Acetone	Aqueous
200	22	17	12
100	19	15	11
50	18	13	10
25	16	9	9
12.5	13	7	6
6.25	9	6	5
3.125	8	3	4
1.56	7	3	3

Table 3: Zones of Inhibition of root extracts of *Andrographispaniculata* on *Pseudomonas rhodesiae* strain YHBT5 165 MG5711.1

Concentrations (mg/ml)	Zones of Inhibition (mm)		
	Ethanol	Acetone	Aqueous
200	18	17	11
100	17	14	10
50	14	12	9
25	11	9	7
12.5	9	7	7
6.25	8	4	3
3.125	7	3	–
1.56	6	3	–

Table 4: Zones of Inhibition of root extracts of *Andrographispaniculata* on *Acaligenesfaecalis* strain 2 MN636316.1

Concentrations (mg/ml)	Zones of Inhibition (mm)		
	Ethanol	Acetone	Aqueous
200	20	16	10
100	18	15	7
50	16	12	5
25	14	10	–
12.5	13	9	–
6.25	11	6	–
3.125	9	5	–
1.56	6	3	–

Table 5: Zones of Inhibition of root extracts of *Andrographispaniculata* on *Acaligenesfaecalis* strain N148 JQ900529.1

Concentrations (mg/ml)	Zones of Inhibition (mm)		
	Ethanol	Acetone	Aqueous
200	17	14	9
100	16	12	7
50	12	10	5
25	9	8	–
12.5	8	7	–
6.25	7	5	–
3.125	6	4	–
1.56	4	3	–

Table 6: Zones of Inhibition of root extracts of *Andrographispaniculata* on *Acaligenesfaecalis* strain 3 MN636317.5

Concentrations (mg/ml)	Zones of Inhibition (mm)		
	Ethanol	Acetone	Aqueous
200	19	17	7
100	18	14	5
50	15	12	4
25	11	10	3
12.5	10	9	–
6.25	8	7	–
3.125	7	5	–
1.56	6	3	–

Table 7: Minimum Inhibitory Concentration of ethanol root extract of *Andrographispaniculata* on the tested isolates

Test Organisms	Concentrations (mg/ml)								
	200	100	50	25	12.5	6.25	3.125	1.56	MIC
*Acinetobacter johnsonii*strain JUQ303	–	–	–	–	–	+	+	+	12.5
*Pseudomonas rhodesiae*strain YHBT5	–	–	–	–	+	+	+	+	25
*Acaligenes faecalis*strain 2	–	–	–	+	+	+	+	+	50
*Acaligenes faecalis*strain N148	–	–	–	+	+	+	+	+	50
*Acaligenesfaecalis*strain 3	–	–	–	–	–	+	+	+	12.5

Table 8: Minimum Inhibitory concentration of acetone root extract of *Andrographispaniculata* on the tested isolates

Test Organisms	Concentrations (mg/ml)								
	200	100	50	25	12.5	6.25	3.125	1.56	M/C
Acinetobacter johnsonii strain JUQ303	–	–	–	–	+	+	+	+	25
Pseudomonas rhodesiae strain YHBT5	–	–	+	+	+	+	+	+	100
Acaligenes faecalis strain 2	–	–	–	+	+	+	+	+	50
Acaligenes faecalis strain N148	–	–	–	–	+	+	+	+	25
Acaligenes faecalis strain 3	–	–	+	+	+	+	+	+	100

Table 9: Minimum Inhibitory concentration of aqueous root extract of *Andrographispaniculata* on the tested isolates

Test Organisms	Concentrations (mg/ml)								
	200	100	50	25	12.5	6.25	3.125	1.56	MIC
Acinetobacter johnsonii strain JUQ303	–	–	+	+	+	+	+	+	100
Pseudomonas rhodesiae strain YHBT5	–	+	+	+	+	+	+	+	200
Acaligenes faecalis strain 2	–	+	+	+	+	+	+	+	200
Acaligenes faecalis strain N148	–	–	+	+	+	+	+	+	100
Acaligenes faecalis strain 3	–	+	+	+	+	+	+	+	200

Table 10: Minimum Bacterial concentrations of *Andrographispaniculata* root extracts on the tested isolates

Test Organisms	Minimum Bacterial Concentration (Mg/ml)		
	Ethanol	Acetone	Aqueous
Acinetobacter johnsonii strain JUQ303	25	12.5	–
Pseudomonas rhodesiae strain YHBT5	12.5	12.5	–
Acaligenes faecalis strain 2	25	25	–
Acaligenes faecalis strain N148	12.5	100	–
Acaligenes faecalis strain 3	12.5	100	–

Table 11: Results of Qualitative Analysis of the Phytochemical screening of the root extracts of *Andrographispaniculata*

Phytochemicals	Percentages
Alkaloids	25.90
Steroids	0.20
Tannins	1.87
Flavonoids	14.50
Saponins	0.71
Andrographolides	41.80
Terpenoids	0.30
Anthracyanin	1.26

Table 12: Results of Qualitative Analysis of the Phytochemical screening of the root extracts of

Phytochemicals	Inferences
Alkaloids	+ +
Phenol	+
Tannins	+ +
Flavonoids	+ + +
Saponin	+ +
Steroids	+
Terpenoids	+
Andrographolide	+ + +
Anthracyanin	+

Key:
+ = Present in trace amount
++ = Moderately high
+++ = Present in high amount

DISCUSSION

The present study was carried out to determine the antimicrobial activity of *Andrographispaniculata* root extracts on *Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* strain YHBT5, *Acaligenesfaecalis* strain 2, *Alcaligenesfaecalis* strain N148 and *Acaligenesfaecalis* strain 3, isolated from patients with diabetic foot ulcers. The ethanol, acetone and aqueous extracts of the roots of *Andrographispaniculata* were evaluated for their antimicrobial potency. It has been found that the various extracts used revealed effective antimicrobial properties of the roots of *Andrographispaniculata* against the tested organisms above. The antimicrobial susceptibility pattern of the ethanol extracts of *Andrographispaniculata* root extract showed that the ethanol extract had more inhibitory effect on the tested organisms than the acetone and aqueous extracts but at varying concentrations.

At the concentration of 200mg/ml, the tested organisms: *Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* strain YHBT5, *Acaligenesfaecalis* strain 2, *Alcaligenesfaecalis* strain N148 and *Acaligenesfaecalis* strain 3 were susceptible to the ethanol root extract having their zones of inhibition recorded as 22mm, 18mm, 20mm,17mm and 19mm respectively at the same concentration (Tables 2, 3, 4, 5, and 6). At lower concentration of 3.125mg/ml and 1.56mg/ml, the above tested organisms showed little susceptibility effect to the ethanol root extract having their zones of inhibition recorded as 8mm, 6mm, 6mm, 4mm and 6mm for *Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* strain YHBT5, *Acaligenesfaecalis* strain 2, *Alcaligenesfaecalis* strain N148 with the and *Acaligenesfaecalis* strain 3 respectively (Tables 2, 3, 4, 5, and 6).

The above results revealed that ethanol root extract of *Andrographispaniculata* had more antimicrobial effect on the tested organisms at higher concentrations. Therefore, the above results in turn agreed with the findings of Kadhim et al. (2020).

The acetone extracts of *Andrographispaniculata* also showed appreciable effect on the tested isolates having inhibition zones of 17mm, 17mm, 16mm, and 14mm zones of inhibition at the concentration of 200mg/ml on *Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* strain, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes faecalis* strain 3 respectively (Tables 2,3,4,5, and 6). The inhibitory effects were seen more in higher concentrations of 200mg/ml, 100mg/ml 50mg/ml and 25mg/ml (Table 2, 3 and 4). At lower concentrations the acetone leaves extract showed less inhibitory activities on the tested isolates. The above result also agrees with the works of Govind and Madhuri, (2018). The results of the aqueous extracts of *Andrographispaniculata* root showed less inhibitory effect on the tested isolates. The study

revealed that *Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* strain YHBT5, *Acaligenesfaecalis* strain 2, *Alcaligenesfaecalis* strain N148 and *Acaligenesfaecalis* strain 3 were susceptible to the plant extracts only at higher concentrations of 200mg/ml and 100mg/ml (Table 2, 3 and 4). At lower concentrations of 12.5, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml, the tested isolates were resistant to the aqueous extracts (Table 3, 4, 5, and 6). The above result also conforms with the works of Abutbulet al. (2018) which stated that *A. johnsonii* and *P. rhodesiae* have their inhibitory effects only at higher concentrations giving their zones of inhibition as 20mm at the highest concentration of 2000mg/ml.

The MIC values reported on the ethanol, acetone and aqueous extracts of *A.paniculata* leaves were carried out on *A.johnsonii* strain JUQ303, *P.rhodesiae* strain YHBT5, *A.faecalis* strain 2, A. *faecalis* strain N148 and *A.faecalis* strain were found to be potent. The MIC of the ethanol, acetone and aqueous extracts were determined for the isolates mentioned above at varying concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml respectively using the agar well dilution method. The MIC value is the lowest concentration that completely inhibited the growth of the microorganisms grown aseptically. The MIC results for the ethanol root extract of *A.paniculata* were recorded at 12.5mg/ml, 25mg/ml, 50mg/ml, 50mg/ml and 100mg/ml forA.*johnsonii* strain JUQ303, *P.rhodesiae* strain YHBT5, *A.faecalis* strain 2, *A.faecalis* strain N148 and *A.faecalis* strain 3, respectively (tables 7). The above results indicated that the potency of the ethanol root extract of *A.paniculata* to the isolates was seen at higher concentrations than the lower concentrations.

The MIC values for the acetone root extract of *Andrographispaniculata* were recorded at 25mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 100mg/ml for *Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* YHTB5, *Acaligenesfaecalis* strain 2, *Alcaligenesfaecalis* strain YHBT5 N148 and *Acaligenesfaecalis* strain 3, respectively (Table 8).

The MIC values for the aqueous extract of *Andrographispaniculata* leave on the tested isolates were found to be potent only at higher concentrations of 100mg/ml, 200mg/ml, 200mg/ml, 100mg/ml and 200mg/ml for*Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* strain YHBT5, *Acaligenesfaecalis* strain 2, *Alcaligenesfaecalis* strain N148 and *Acaligenesfaecalis* strain 3 respectively (Table 9). The above results of the aqueous extract of *Andrographispaniculata* on the tested isolates conforms with the findings of Ganeshmurthy et al. (2016).

The MBC result for the ethanol root extract of *Andrographispaniculata* were recorded at 25mg/ml, 12.5mg/ml, 25mg/ml 12.5mg/ml and 12.5mg/ml while that of acetone root extract were recorded at125.mg/ml, 12.5mg/ml,25mg/ml, 100mg/ml and 100mg/ml for *Acinetobacterjohnsonii* strain JUQ303, *Psedomonasrhodesiae* strain YHBT5, *Acaligenesfaecalis* strain 2, *Alcaligenesfaecalis* strain N148 and *Acaligenesfaecalis* strain 3 respectively (Table 10). The result of the aqueous rootextract of *Andrographispaniculata* had no bactericidal effect on the tested isolates.

The results of the phytochemical screening of the root of *Andrographispaniculata* extracts revealed the presence of Alkaloids, Saponins, Andrographolides, Tannins, flavonoids, steroids and Terpenoids (Table 12). The results obtained from the phytochemicals and micronutrient screening of *Andrographispaniculata* gives credence to the medicinal benefits that this herb have been used for in the past years and supports its traditional use for the management of various health problems. These results above suggest that the antimicrobial activity shown by the extracts against the tested isolates might be due to its natural occurring bioactive phytochemicals present in the plant Hosamani et al. (2017). This suggests that the plant could serve as a remedy to fight against infections caused by pathogens of wound origin. It has been widely observed and accepted that the medicinal values of plants are dependent on the bioactive phytochemicals present in plants, this research study has brought to knowledge, the promising benefits of exploring Andrographis paniculata plant for its antimicrobial potentials and other medicinal values.

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