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Phytochemical and Antibacterial Screening of Andrographis paniculata (King of Bitterness) Leaf Extracts on Bacteria Isolated from the Foot Ulcers of Diabetic Patients

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Abstract:

Foot ulcers are common among diabetic patients and can lead to severe bacterial infections. The aim of the study was to determine the antibacterial activity of Andrographis paniculata leaf 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 Alcaligene faecalis strain 3 were isolated from patients attending clinics at the National Orthopedic Hospital, Enugu. The leaves of Andrographis paniculata were obtained from a local garden in Anaocha, a local government area in Anambra state. The leaves of Andrographis paniculata were pulverized and extracted using ethanol, acetone and aqueous solvents. Preliminary qualitative and quantitative phytochemical analyses of the leaf extracts were done using standard methods to reveal the presence of the concentrations of basic phytocomponents.

The ethanol, acetone and aqueous crude extracts of the leaves of Andrographis paniculata were reconstituted using 5ml of Dimethyl sulfoxide (DMSO) to obtain concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56mg/ml. The isolates were screened for sensitivity to the plant extracts using the agar well diffusion method. The minimum inhibitory concentration was determined by the agar well diffusion method. The antibiotic susceptibility pattern of the isolates was analyzed using the Kirby-Bauer disc diffusion method. The phytochemical analysis of the ethanol, acetone and aqueous extracts of the leaves of Andrographis paniculata showed the presence of phytochemicals (alkaloids, phenols, tannins, flavonoid, saponin, steroid, terpenoid, andrographolide and anthracyanin). Quantitative analysis of the extracts showed varied concentrations of the phytocomponents. At 200mg/ml, the ethanol and acetone extracts of Andrographis paniculata showed higher zones of inhibition on all the isolates. Ethanol extracts of the leaves showed the highest zones of inhibition at 25mm on Acinetobacter johnsonii strain JUQ303, 23mm on Pseudomonas rhodesiae strain YHBT5, 22mm on Acaligenes faecalis strain 2, 19mm Acaligenes faecalis strain N148 and 21mm on Acaligenes faecalis 3, respectively. The aqueous extracts showed the highest zone of inhibition at 14mm on Acinetobacter johnsonii, 12mm on Pseudomonas rhodesiae, 13mm on Alcaigenes faecalis strain 2, 10mm on Alcaligenes faecalis strain N148 and 9mm 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 the tested isolates. The MIC of the ethanol extract of Andrographis paniculata leaves were recorded at 25mg/ml,12.5mg/ml, 50mg/ml, 25mg/ml and 100mg/ml. The MIC of the acetone extracts of Andrographis paniculata leaves were recorded at 50mg/ml, 25mg/ml,100mg/ml, 12.5mg/ml and 12.5mg/ml, while the MIC for the aqueous extract was recorded at 100mg/ml, 200mg/ml,100mg/ml, 100mg/ml and 100mg/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 MBC of the ethanol and acetone extract was recorded at 25mg/ml and 12.5mg/ml for Acinetobacter johnsonii, 12.5 and 12.5 for Pseudomonas rhodesiae, 25mg/ml and 25mg/ml for Alcaligenes faecalisstrain 2, 12.5mg/ml and 100mg for Alcaligenes faecalis strain N148 and 12.5mg/ml and 100mg/ml for Alcaligenes faecalis strain 3, respectively. The aqueous extract showed no bactericidal effect on the tested isolates. From the study, ethanol extracts of the leaves of Andrographis paniculata showed the highest antibacterial potency on the 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: Andrographis paniculata, phytochemical, Antimicrobial, Acinetobacter johnsonii, Pseudomonas rhodesiae, Alcaligenes faecalis

1. Introduction

Plants produce a wide range of bioactive molecules, making them a 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 the pharmaceutical field, medicinal plants are largely used for a broad range of substances present in plants, which have been used to treat infectious and 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 synthetic drugs have side effects. Also, most 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, are less toxic, scanty, and 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 a potentially beneficial alternative and a 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 the "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. It prefers to grow well in a diversity of habitats such as moist, shady areas, hill slopes, plains, farms, seashores, wastelands and dry or wetlands (Hosamani et al., 2017). It is rich in a wide variety of phytochemical constituents such as Diterpens, Flavonoids and Lactones (Kadhim et al., 2020).

Andrographis paniculata is extensively used in Ayurveda, Unani and Siddha medicine as a home remedy for various diseases in India's traditional system and in tribal medicine applications. The therapeutic value of Andrographis paniculata is due to its mechanism of action by enzyme induction. It is a powerful cold property herb used to control fever, sore throat, hepatitis and a variety of other chronic and infectious diseases. (Govind and Madhuri, 2018). The herb and its isolates, such as isoandrographolide, neoandrographolide, and andrographolide, are reported to possess anti-inflammatory activity, hepatoprotective, anti-diabetic, antimalarial, and antimicrobial activities (Dhawan, 2018).

The plant has been reported to also possess immuno-stimulatory ability, anti-cancer, arresting dysentery, cholera, influenza, bronchitis, swelling and itches, piles and gonorrhea when prepared in tonics (Sukanya *et al.*, 2019). Herbal drugs in disease management attain success because they are cost-effective, eco-friendly and have minimal side effects (Punitha *et al.*, 2018).

Diabetes mellitus is a metabolic, endocrine disorder caused by an overall deficiency of insulin (type 1 diabetes) or defective insulin function (type 2 diabetes), which causes hyperglycemia (Tepe *et al.*, 2018).

Diabetic foot ulcers are among the most common complications of patients who have diabetes mellitus, which is not well controlled (Yu *et al.*, 2018). It is usually the result of poor glycemic control, underlying neuropathy, peripheral vascular disease, or poor foot care. It is also one of the common causes of Osteomyelitis. These ulcers are usually in areas of the foot which encounter repetitive trauma and pressure sensation resulting from microbial infections (Wang *et al.*, 2017). Diabetic foot ulcers are responsible for more admissions than any other diabetes complication. Today, diabetes is the leading cause of non-traumatic amputations in the world. Overall, about 25% of patients with diabetes mellitus develop foot ulcers, and 5% end up with an amputation, which is a result of a progressive infection caused by pathogenic bacteria which are resistant to therapeutic drug treatment regimens Zaiden *et al.*, (2018).

2. Materials and Methods

2.1. Collection and Processing of Plant Material

The leaves of the plant *Andrographis paniculata* 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 leaves were washed thoroughly with distilled water and dried for 7 days at room temperature. The dried leaves were blended to powder with the aid of a sterile blender and were stored in an air-tight container until required for the analysis.

2.2. Preparation of Plant Extracts

A modified method of Abdulrahman *et al.* (2014) was used. Fifty (50) grams of the ground leaves of *Andrographis paniculata* were weighed into three conical flasks containing 200mls of solvent extractants (Aqueous, Acetone and Ethanol), respectively. The conical flasks were covered tightly and left for 48 hours to extract at 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 4°C in a refrigerator until used for experimentation.

2.3. Standardization of Inoculum

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 the antimicrobial susceptibility test.

2.4. Antimicrobial Susceptibility Test Using the Extracts

The agar well diffusion method was used to determine the antibacterial activity of the extracts.

To test for this, 1g of each of the extracts was dissolved in 5 ml 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 1.5×10^8 cells (*Acinetobacter johnsonii strain JUQ303, Pseudomonas rhodesiaestrain YHBT5, Alcaligenes faecalis strain 2, Alcaligenes faecalis strain N148 and Alcaligene 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 in the Mueller. Hinton agar plates in which 0.1ml of various concentrations of the extracts were added. The plates were then incubated at 37° C for 24 hours, and the zones of inhibition were measured.

2.5. Determination of the Minimum Inhibitory Concentrations (MICs)

This was determined using the agar well dilution method. 0.1ml of the inoculum(*Acinetobacter johnsonii strain JUQ303*, *Pseudomonas rhodesiae strain 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, 1.562mg/ml and 0.76mg/ml) were added into each of the wells respectively and they were incubated at 37°C for 24 hours. After the incubation, the MIC was determined to be the least concentrated extract that inhibited the growth of the organism.

2.6. Determination of Minimum Bacterial Concentrations (MBCs)

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° C for 24 hours.

Test tubes with no visible growth were streaked on various plates containing Mueller Hinton agar and incubated at 37°C for 24 hours. 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.

2.7. Phytochemical Screening of the Plant Extract

The leaf extracts were screened for their phytochemical activity (qualitative and quantitative analysis) using the standard method. The phytochemical components analyzed were Alkaloids, Flavonoids, Tannis, Saponins, Phenol, Steroids and Terpenoids.

3. Results

Isolates	Strain	Accession Number
Acinetobacter johnsonii	JUQ303	MN826149.1
Pseudomonas rhodesiae	YHBT5	MG571711.1
Acaligenes faecalis	2	MN636316.1
Acaligenes faecalis	N148	JQ900529.1
Acaliaenes faecalis	3	MN636317.1

Table 1: The Result of the Isolates, the Strains and Accession Numbers

Concentrations (mg/ml)	Zones	Zones of Inhibition (mm)				
	Ethanol	Acetone	Aqueous			
200	25	18	14			
100	23	15	13			
50	21	10	10			
25	20	9	7			
12.5	17	7	6			
6.25	13	6	5			
3.125	10	6	3			
1.56	7	5	_			

Table 2: Zones of Inhibition of Leaf Extracts of Andrographis paniculata on Acinetobacter johnsonii Strain JUQ303 MN826149.1

Concentrations (mg/ml)	Zones of Inhibition (mm)						
	Ethanol	Acetone	Aqueous				
200	23	16	12				
100	21	15	11				
50	20	14	8				
25	18	11	7				
12.5	16	9	6				
6.25	14	7	4				
3.125	11	6	3				
1.56	7	3	3				

Table 3: Zones of Inhibition of Leaf Extracts of Andrographis paniculata on Pseudomonas rhodesiae Strain YHBT5 MG5711.1

Concentrations (mg/ml)	Zones of Inhibition (mm)						
	Ethanol	Acetone	Aqueous				
200	22	19	13				
100	20	17	11				
50	18	14	8				
25	17	13	6				
12.5	15	11	4				
6.25	12	9	_				
3.125	9	6	-				
1.56	7	5	-				

Table 4: Zones of Inhibition of Leaf Extracts of Andrographis paniculata on Acaligenes faecalis Strain 2 MN636316.1

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

Table 5: Zones of Inhibition of Leaf Extracts of Andrographis paniculata on Acaligenes faecalis Strain N148 JQ900529.1

Concentrations (mg/ml)	Zones of Inhibition (mm)						
	Ethanol	Acetone	Aqueous				
200	21	15	9				
100	20	14	7				
50	18	11	5				
25	16	10	3				
12.5	12	8	_				
6.25	10	6	_				
3.125	9	5	_				
1.56	6	4	_				

Table 6: Zones of Inhibition of Leaf Extracts of Andrographis paniculata on Acaligenes faecalis Strain 3 MN636317.5

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

Table 7: Minimum Inhibitory Concentration (MIC) of Andrographis paniculata of Ethanol Leaf Extracts on the Tested Isolates

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

Table 8: Minimum Inhibitory Concentration (MIC) of Andrographis paniculata of Acetone Leaves Extract on the Tested Isolates

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

Table 9: Minimum Inhibitory Concentration (MIC) of Andrographis paniculata of Aqueous Leaf Extracts on the Tested Isolates

Test Organisms	Minimum Bacterial Concentration (mg/ml)					
Acinetobacter johnsonii strain JUQ303	Ethanol	Acetone	Aqueous			
Pseudomonas rhodesiae strain YHBT5	100	25	_			
Acaligenes faecalis strain 2	12.5	12.5	_			
Acaligenes faecalis strain N148	200	25	_			
Acaligenes faecalis strain 3	50	12.5	_			
	100	50	_			

Table 10: Minimum Bacterial Concentrations of Andrographis paniculata Leaf Extracts on the Tested Isolates

Phytochemicals	Percentag	es	
Alkaloids	63.75		
Steroids	0.50		
Tannins	4.57		
Flavonoids	25.01		
Saponins	2.87		
Andrographolides	53.10		
Terpenoids	0.30		
Anthracyanin	3.32		

Table 11: Results of Qualitative Analysis of the Phytochemical Screening of the Leaf Extracts of Andrographis paniculata

Phytochemicals	Inferen	ce	
Alkaloids	+++		
Phenol	+		
Tannins	++		
Flavonoids	+++		
Saponin	+		
Steroids	+		
Terpenoids	+		
Andrographolide	++		
Anthracyanin	++		

Table 12: Results of Qualitative Analysis of the Phytochemical Screening of the Leaf and Root Extracts of Andrographis paniculata

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Key:

- += Present in trace amount
- ++= Moderately high

+ + += Present in high amount.

4. Discussion

The present study was carried out to determine the antimicrobial activity of *Andrographis paniculata* leaf extracts on *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenesfaecalis* strain 3, isolated from patients with diabetic foot ulcers. The ethanol, acetone and aqueous extracts of the leaves of *Andrographis paniculata* were evaluated for their antimicrobial potency. It has been found that the various extracts used revealed effective antimicrobial properties of the leaves of *Andrographis paniculata* against the tested organisms above. The antimicrobial susceptibility pattern of the ethanol extracts of *Andrographis paniculata* leaves extract showed that the ethanol extract had a 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: *Acinetobacter johnsonii* strain JUQ303 with *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes faecalis* strain 3 were susceptible to the ethanol leaves extract having their zones of inhibition recorded as 25mm, 23mm, 22mm,19mm and 21mm respectively at the same concentration (Tables 2, 3, 4, 5 and 6). At lower concentrations of 3.125mg/ml and 1.56mg/ml, the above-tested organisms showed little and no appreciable susceptibility effect to the ethanol leave extract having their zones of inhibition recorded as 7mm, 7mm, 7mm, 3mm and 6mm for *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes faecalis* strain 3, respectively (Tables 2, 3, 4, 5 and 6).

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

The acetone extracts of *Andrographis paniculata* also showed an appreciable effect on the tested isolates having inhibition zones of 18mm, 16mm, 19mm, 12mm and 15mm zones of inhibition at the concentration of 200mg/ml on *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* 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 (Tables 2, 3 and 4). At lower concentrations, the acetone leaf extract showed less inhibitory activity on the tested isolates. The above result also agrees with the works of Govind and Madhuri (2018). The results of the aqueous extracts of *Andrographis paniculata* leaves showed less inhibitory effect on the tested isolates. The study revealed that *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes faecalis* strain 3 were susceptible the plant extracts only at the higher concentrations of 200mg/ml and 100mg/ml (Tables 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 (Tables 3, 4, 5 and 6). The above result also conforms to the works of Abutbul *et al.* (2018), which stated that *Acinetobacter johnsonii* and *Pseudomonas 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 *Andrographis paniculata* leaves were carried out on *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes 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 broth dilution method. The MIC value is the lowest concentration, which completely inhibited the growth of the microorganisms grown aseptically. The MIC results for the ethanol leaves extract of *Andrographis paniculata* were recorded at 25mg/ml, 12.5mg/ml, 50mg/ml, 25mg/ml and 100mg/ml for *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes faecalis* strain 3, respectively (Table 7). The above results indicated that the potency of the ethanol leaves extract of *Andrographis paniculata* to the isolates was seen at higher concentrations than the lower concentrations.

The MIC values for the acetone leave extract of *Andrographis paniculata* were recorded at 50mg/ml, 25mg/ml, 100mg/ml, 12.5mg/ml and 12.5mg/ml (Table 8) for *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* YHTB5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain YHBT5 N148 and *Acaligenes faecalis* strain 3, respectively.

The MIC values for the aqueous extract of *Andrographis paniculata* left on the tested isolates were found to be potent only at higher concentrations of 100mg/ml, 200mg/ml, 100mg/ml, 100mg/ml and 100mg/ml (Table 9) for *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes faecalis* strain 3, respectively. The above results of the aqueous extract of *Andrographis paniculata* on the tested isolates conform with the findings of Ganeshmurthy *et al.* (2016).

The MBC result for the ethanol leaves extract of *Andrographis paniculata* were recorded at 100mg/ml, 12.5mg/ml, 200mg/ml, 50mg/ml and 100mg/ml (Table 10), while that of acetone leaves extract were 25mg/ml, 12.5mg/ml, 12.5mg/ml, 12.5mg/ml and 50mg/ml for *Acinetobacter johnsonii* strain JUQ303, *Pseudomonas rhodesiae* strain YHBT5, *Acaligenes faecalis* strain 2, *Alcaligenes faecalis* strain N148 and *Acaligenes faecalis* strain 3, respectively. The MBC result for the ethanol root extract of *Andrograhis paniculata* showed an appreciable effect, having its bactericidal concentration recorded at 25mg/ml, 12.5mg/ml, 25mg/ml, 12.5mg/ml and 12.5mg/ml (Table 10). The result of the aqueous extract of *Andrographis paniculata* leaves had no bactericidal effect on the tested isolates.

The results of the phytochemical screening of the leaves and root of *Andrographis paniculata* 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 *Andrographis paniculata* give credence to the medicinal benefits that this herb has been used for in the past years and support its traditional use for the management of various health problems.

The results above suggest that the antimicrobial activity shown by the extracts against the tested isolates might be due to the naturally 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 the plants; this research study has brought to knowledge the promising benefits of exploring *Andrographis paniculata* plant for its antimicrobial potentials and other medicinal values.

5. References

- i. Abdulrahman, H. L., Sudisha, J., Hariprasad, P., Miranjama, S. R., Prakash, H. S., & Fathima, S. K. (2019). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, 8(23), 6677–6682.
- ii. Abutbul, S., Golan-Goldhirsh, A., Barazani, O., Ofir, R., & Ziberg, D. (2018). Screening of desert plants for use against bacterial pathogens in fish. *Israeli Journal of Aquaculture*, *57*(2), 71–80.
- iii. Ganeshmurthy, R., Ajith-Kumar, T. T., & Dhayanithi, N. B. (2016). Effect of secondary metabolites of the seaweed (Halimeda Micronesia) at Lakshadweep Islands against aquatic pathogens. *International Journal of Pharmaceutical and Biosciences*, 3(2), 213–220.
- iv. Govind, P., & Madhuri, S. (2018). Significance of fruits and vegetables in malnutrition cancer. *Journal of Plant Archives*, 10(2), 517–522.
- v. Hosamani, P. A., Lakshman, H. C., Kumar, S. K., & Rashimi, C. (2017). Antimicrobial activity of leaf extract of Andrographis paniculata wall against clinical pathogens. *Journal of Science Research*, 1(2), 92–95.
- vi. Kadhim, W. A., Kadhim, M. J., & Hameed, I. H. (2020). Antibacterial activity of several plant extracts against proteus species. *International Journal of Pharmaceutical and Clinical Research*, 8(12), 1673–1684.
- vii. Okigbo, R. N., Anuagasi, C. L., & Amadi, J. E. (2019). Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medicinal Plants Research*, 3(2), 86–95.
- viii. Punitha, S. M. J., Babu, M. M., Sivaram, V., Shankar, V. S., & Mahash, T. C. (2018). Immuno stimulating influence of herbal biomedicines on non-specific immunity in grouper Epinephelus tauvina against vibrio harvaji infection. *Journal of Aquaculture*, 16, 511–523.
 - ix. Sridevi, M., Kandala, R. B., & Sathiraju, D. (2016). Sensitivity of bacterial isolated from champearaithi estuary to some medicinal plants of Vizinagaramn district, East Coast of India. *Journal of Drug Invention*, *2*(7), 366–368.
 - x. Stefanello, M. E. A., Cervi, A. C., Ito, I. Y., & Wisniewski, A. (2018). Chemical composition and antimicrobial activity of essential oils of Eugenia Chlorophylla (Myrtaceae). *Journal of Essential Oils Research*, 20(1), 75–78.
- xi. Sukanya, S. L., Sudisha, J., Hariprasad, P., Miranjama, S. R., Prakash, H. S., & Fathima, S. K. (2019). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, 8(23), 6677–6682.
- xii. Tajikarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2017). Antimicrobial herb and spice compounds in food control. *Journal of Biology, 21*(9), 1199–1218.
- xiii. Tepe, B., Daferera, D., & Sekmen, A. (2018). In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of Thymus eigii. *Journal of Agricultural and Food Chemistry*, *52*, 1132–1137.
- xiv. Wang, Z. T., & Ji, L. L. (2017). In vivo and in vitro anti-inflammatory activities of neoandrographolide. *The American Journal of Chinese Medicine*, 35, 317–328.
- xv. Yu, B. C., Hung, C. R., & Cheng, J. T. (2018). Anti-hyperglycemic effect of andrographolide in streptozotocin-induced diabetic rats. *Journal of Plants Medicas*, 69, 1075–1079.
- xvi. Zaiden, M. R., Rahul, K. S., & Shree, P. F. (2018). Antimicrobial activity of Andrographis Paniculata (Burm.f) leaves against clinical pathogens of fecal origin. *Journal of Pharmacy Research*, 7, 459–462.