International Conference on Sciences, Engineering & Environmental Technology (IJOSEET), 6(39): 311– 316, 2021
ISSN 0794-9650

www.repcomseet.org

Phytochemical Studies and Antimicrobial Activities of King of Bitters (*Andrographis Paniculata*) Against Some Selected Microorganisms

Ajayi, O.A. (PhD)[a*], Siyanbola, G. A.[b] , Akanfe, F. A[c]. and Owonibi, S.K[d]

[a-d] Science and Laboratory Technology Department
Federal Polytechnic Ede, Osun State. Nigeria
Correspondence: oyinajayiforsuccess@gmail.com.

Abstract: The emergence of antibiotic resistance pathogenic microbes coupled with the side effects of antibiotics have necessitated the search for other alternative and newer antimicrobial agents that are mainly of natural sources. Andrographis paniculata (king of bitters) is one of the natural sources of antimicrobial agents. The ethanolic and aqueous extracts of the different parts of king of bitters were screened against selected pathogenic microorganisms using agar well diffusion method while the phytochemical constituents were determined by spectrophotometric methods. Aqueous and ethanolic extracts from the different parts of king of bitters demonstrated antibacterial activities against E. coli, S. aureus and S. dysenteriae with the zone of inhibition ranging from 5 mm to 15 mm and 9 mm to 35 mm respectively. Also, the aqueous and ethanolic extracts from the different parts of king of bitters exhibited appreciable antifungal activities against Aspergillus fumigatus with the zone of inhibition ranging from 10 mm to 40 mm and 15 mm to 53 respectively. The minimum inhibitory concentrations of the extracts from the different parts ofking of bitters was between 3000 - 5000 µg/ml for the bacteria strains and 2000 - 3000 µg/ml for the fungus. Phytochemical analysis of the extracts revealed the presence of phenol, tannins, saponin, flavonoids and alkaloids in different concentrations. Thus, the study supports the use of extracts from the different parts of this plants in treatment of various infections and recommend the use of the leaf extracts at 3000-5000 µg/ml concentration.

Keywords: *Andrographis paniculata*, anti-microbial, aqueous extract, ethanolic extract and phytochemical.

1. Introduction

Medicinal plants have been used over the years to treat various type of acute and chronic diseases. There is a growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Kokate, *et al.* 2003, Ajayi *et al*., 2011). Medicinal plants contain a lot of bioactive constituents or phytochemical compounds which are secondary metabolites that produced by plant. The World Health Organization (WHO) opines that about 80% of African population are dependent on the traditional herbal medicines for their primary health care needs (Poongothai, *et al.,* 2011). The practice of transmitting healing knowledge from generation to generation has enabled the consistency of application of given herbs for specific ailments.

Andrographis paniculata commonly known as "king of bitter" belongs to family Acanthacea, is an important annual medicinal herb widely distributed in Madhya Pradesh, India. It is hardy and erect herb which grows mainly as under shrub in tropical, moist deciduous forest. It is one of the most widely used plant in Ayurvedic formulations (Poongothai, *et al.,* 2011). It is cultivated because of its well-known medicinal value, and it grows well in most soil types, thus it is widely distributed. The aerial parts and roots of *Andrographis* are have been widely used as traditional medicine to treat many diseases in China, India, Thailand and other Southeast Asian countries. Such diseases include intermittent fevers, pyrexia, dyspepsia, influenza, dysentery, malaria respiratory infections and stomach aches. The entire plant extract has been used for several applications such as antidote for snake-bite and poisonous stings of some insects (Ajayi *et al*., 2011, Kumar *et al*., 2012).

Thus, the present study aimed at comparative analysis and antimicrobial activities of the extracts of the different parts of *Andrographis paniculata* against common pathogenic microorganisms in order to authenticate the efficacy of using this plant for treating common ailments and to ascertain the required concentrations.

2.0 MATERIALS AND METHOD

2.1 Collection of Plant Material

The healthy and fresh plant of *Andrographis paniculata* was collected from Iwo, Osun - State. The identification of the plant sample was confirmed at the Herbarium unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife. The plant was separated into leaves stems and roots. The different parts were washed and under tap water for 3 times and allowed to drain.

2.2 Collection and Maintenance of Test Organisms

Pure clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Aspergilus fumigatus* were collected from the Microbiology Unit of the Science Laboratory Technology Department, Federal Polytechnic, Ede. The bacteria were maintained on nutrient agar while the fungus was maintained on potato dextrose agar and stored at 4ºC.

2.3 Preparation of Extracts

Aqueous and ethanolic extracts were prepared using the procedure of Olajire and Azeez (2011) with little modification. Portions (30 g) each of the different parts of the plants were mashed and blended with 100 ml of distilled water and ethanol respectively. The mixture was placed in a clean conical flask in which 200 ml of distilled water and ethanol were added respectively to make up 300 ml of the solvents. The mixtures were allowed to stand for 72 hours at 37ºC. The mixtures were filtered using muslin cloth and the filtrate was collected into sterile container. The filtrates were centrifuged at 2000 rpm for 5 minutes. The residues were discarded while the filtrates were poured into a sterile container.

2.4 Sterility Test of the Plant

Each of the extracts was tested for the growth of contaminants according to the method of Akinpelu and Kolawole (2007). A portion (1 ml) of each of the extracts was inoculated on sterile nutrient agar and potato dextrose agar. The nutrient agar plates were incubated at 37ºC for 24 hours while the potato dextrose agar plates were incubated at 27º C for 72 hours. After incubation, the plates were observed for growth of contaminants.

2.5 Antimicrobial Activities of extracts of the different parts of Andrographis paniculata

The microbial properties of the aqueous and ethanolic extracts of different parts of *A. paniculata* were determined using agar well diffusion method as described by Cheesbrough (2004). Nutrient was used for the bacteria isolates while Potato Dextrose Agar was used for the fungus. An aliquot (0.1 ml) of the test isolate was spread on the agar plate using glass spread. A sterile stainless-steel cork-borer was used to make holes (8 mm in diameter) on the medium which has been seeded with the test organism. An aliquot (0.1 ml) of each extract was dropped into the well. The procedure was repeated for all the extracts with different organism. Distilled water, ethanol and standard antibiotics (Metronidazole and fluconazole at 10 mg/ml) were used as the control. The experiments were done in triplicates. The bacteria plates were incubated at 37°c and the zones of inhibition was measured after for 24 hours after while the fungal plates were left at room temperature. The zones of inhibition were measured after 24 hours and 72 hours respectively for the bacteria and fungus plates.

2.6 Determination of Minimum Inhibitory Concentration (MIC) of extracts different parts of A. paniculate

The MIC was determined by using the method of Akinpelu and Kolawole (2004). The concentrations of the extracts used for the MIC are 10 mg/ml, 5 mg/ml and 2 mg/ml. Two-fold dilution of each of crude extracts was prepared and 2 ml of different concentrations of the solution was added to 18 ml of sterilized molten nutrient agar at 40°c to give final concentration of 10 mg/ml and the procedure was repeated to give concentration of 5 mg/ml and 2 mg/ml. The media were then poured into sterile petridishes and were allowed to set. The media plates were streaked with 17 hours old culture, and the plates were incubated at 37 °C for 24 hours for bacteria and 27 °C for 72 hours for fungus

2.6 Phytochemical Studies

2.6.1 Determination of total phenolic compounds

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu's reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. Gallic acid was used as a standard. This assay was carried out in triplicate.

2.6.2 Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminum complex which has an absorptivity maximum at 415nm. 100 µl of the sample extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of sample extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions. This assay was carried out in triplicate.

2.6.3 Determination of total alkaloids:

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid. The calculation was then made.

2.6.4 Determination of total tannins

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm within 10 minutes. Blank sample was prepared and subjected to the same procedure. A standard was prepared by using tannin acid.

2.6.5 Determination of total saponins

The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The Samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated.

2.7 Statistical Analyses

The data obtained from the experiments were subjected to two-way Analysis of Variance (ANOVA) while the mean differences were compared using pairwise comparison and Duncan's New Multiple Range Test (DMRT) at 5 % level of probability.

3.0 RESULTS AND DISCUSSION

The antimicrobial activities of *Andrographis paniulata* against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysentery* and *Aspergillus fumigatus* are presented in Table 1 and 2

TABLE 1: Zones of inhibition of different bacteria and fungi activities of *Andrographis paniculate*

TREATMENTS (10 MG/ML)	*ESCHERICHIA COLI* (ZI IN MM)	*SHIGELLA DYSENTERY* (ZI IN MM)	*STAPHYLOCOCCUS AUREUS* (ZI IN MM)	*ASPERGILLUS FUMIGATUS* (ZI IN MM)
AQUEOUS LEAF EXTRACT	15 ± 0.82^{d}	10 ± 1.63^{c}	10 ± 1.63^{e}	40 ± 1.63^{b}
ETHANOLIC LEAF EXTRACT	35 ± 0.82^{a}	21 ± 0.82^{a}	29 ± 0.82^{a}	53 ± 3.80^{a}
AQUEOUS STEM EXTRACT	13 ± 0.82^{e}	9.5 ± 0.82^{c}	5 ± 0.82^{g}	25 ± 1.72^{d}
ETHANOLIC STEM EXTRACT	20 ± 0.82^{b}	10 ± 0.71^{c}	17 ± 0.82^{c}	35 ± 0.82^{c}
AQUEOUS ROOT EXTRACT	11 ± 0.82^{f}	7 ± 0.82^{d}	7.5 ± 0.82	10 ± 1.63
ETHANOLIC ROOT EXTRACT	15.3 ± 0.47^{d}	9 ± 0.82^{c}	22 ± 0.82^{b}	15 ± 0.82^{e}
METRONIDAZOLE 10	17 ± 0.82^{c}	18 ± 0.82^{b}	13 ± 0.82^{d}	NA
FLUCONAZOLE	NA	NA	NA	15 ± 0.82^{e}
WATER	N.A	N.A	N.A	N.A
ETHANOL	7.2 ± 0.82^{g}	6 ± 0.73^{d}	7.6 ± 0.80^{f}	11 ± 0.54^{f}

KEY: N.A – Not Active, ± - Standard Error (SE), ZI- Zones of inhibition

Values in a column followed by the same letter are not significantly different at $p > 0.05$.

Table 2: Minimum Inhibitory Concentration (μg/ml) of Extracts of Different Parts of *A. paniculata* Against the Test Microorganisms

TREATMENTS	*Escherichia coli* (MIC in μg/ml)	*Shigella dysentery* (MIC in μg/ml)	*Staphylococcus aureus* (MIC in μg/ml)	*Aspergillus fumigatus* (MIC in μg/ml)
Aqueous leaf extract	3000	NA	3000	NA
Ethanolic leaf extract	5000	5000	5000	2000
Aqueous stem extract	NA	3000	NA	3000
Ethanolic stem extract	5000	5000	5000	NA
Aqueous root extract	3000	3000	3000	3000
Ethanolic root extract	5000	5000	5000	3000
Metronidanisole/ Fluconazole	1000	1000	1000	1000

NA: Not active, MIC: Minimum inhibitory concentration

The result of the phytochemical analysis is presented in Table 3.

TABLE 3: Phytochemical constituents (mg/g) of Different Parts of *Andrographis paniculate*

EXTRACTS	PHENOL	ALKALOIDS	TANNINS	FLAVONOIDS	SAPONIN
Aqueous stem extract	41.89±0.03	7.53 ± 0.00	0.19±0.00	2.82 ± 0.02	9.62 ± 0.01
Aqueous root extract	41.98±0.02	5.72 ± 0.02	0.20 ± 0.01	2.88 ± 0.01	9.53 ± 0.02
Aqueous leaf extract	43.79±0.11	9.57 ± 0.01	0.23 ± 0.01	2.90 ± 0.00	9.61 ± 0.01
Ethanolic root extract	43.67±0.04	7.30 ± 0.00	0.23 0.00	3.37 ± 0.05	9.67 ± 0.33
Ethanolic leaf extract	47.81±0.17	9.87 ± 0.20	0.33 ± 0.02	3.67 ± 0.12	10.27±0.04
Ethanolic stem extract	44.83±0.02	8.57 ± 0.00	0.28 ± 0.01	3.17 ± 0.05	9.98 ± 0.02

The result showed that *Escherichia coli* was the most sensitive bacteria to both ethanolic and aqueous leaf extracts with 35 mm and 15 mm zones of inhibition respectively. *Staphylococcus aureus* was also sensitive to both ethanolic and aqueous leaf extracts with 29 mm and 10 mm zones of inhibition respectively while *Shigella dysenteriae* was less sensitive to ethanolic and aqueous leaf extracts with 21 mm and 10 mm zones of inhibition respectively. It was also observed that *Escherichia coli* was the most sensitive bacteria to both ethanoic and aqueous stem extracts with 20 mm and 13 mm zones of inhibition correspondingly. *Staphylococcus aureus* was also sensitive to both ethanolic and aqueous stem extracts with 17 mm and 5 mm zones of inhibition while *Shigella dysenteriae* was less sensitive to the ethanolic and aqueous stem extracts with 10 mm and 9.5 mm zones of inhibition respectively. This result is in contrast to the report of Shakil (2017) who discovered that the stem extract of *Andrographis paniculata* was more sensitive to gram positive bacteria.
Similarly, *Escherichia coli* was the most sensitive bacteria to both ethanoic and aqueous root extracts with 15 mm and 11 mm zones of inhibition respectively while *Staphylococcus aureus* was sensitive to both ethanoic and aqueous root extracts with 22 mm and 7.5 mm zones of inhibition. However, *Shigella dysenteriae* was less sensitive to the ethanolic and aqueous root extracts with 9 mm and 7 mm zones of inhibition respectively. This result was in line with the findings of Suparna *et al*. (2014) who discovered that *Staphyloccocus aureus* was sensitive to the extracts of *A paniculata*. Consequently, it was observed that metronidazole is significantly less sensitive than the extracts with zone of inhibition of 17 mm in *Escherichia coli*,13 mm in *Staphyloccocus aureus* and 18 mm in *Shigella dysenteriae*.
Antifungal test revealed that both ethanolic and aqueous leaf extracts are the most effective against *Aspergillus fumigatus* with zones of inhibition 53 mm and 40 mm respectively. Also, the ethanolic and aqueous stem extracts were also effective with 35 mm and 25 mm zones of inhibition respectively. Fluconazole was less effective against *Aspergillus fumigatus* with 15 mm zone of inhibition. This corroborates the findings of Suparna *et al*. (2014) who reported that leaves showed the best antimicrobial activities.
Phytochemical analysis revealed the presence of secondary metabolites such as tannin, saponin, phenol, flavonoids and alkaloids in the different parts of *Andrographis paniculata* in different concentrations. This indicated that extracts of different parts of the plant are active against strains of organisms tested due to the presence of some secondary metabolites as earlier reported by Ajayi *et al*. (2011) which state that plants that are traditionally claimed to be useful in treating infections produce a lot of phytochemicals.
Ethanolic leaf extract has the highest concentration of phenol, flavonoids and tannin are more concentrated in the leaves than the root while stem aqueous extract has the lowest concentration of phenol, flavonoids and tannin. However, the ethanolic leaf extract has the highest concentration of saponin and alkaloids while root aqueous extract has the lowest concentrations of saponin and alkaloids. This corroborate the findings of Ajayi *et al*., (2018) who reported that phytochemicals are mostly concentrated in the leaves of *Tithonia diversifolia*. However, this negates the findings of Shakil *et al.* (2017) who reported that the metabolites are more concentrated in the stem. This variation might be due to environmental factors.

4.0 CONCLUSION AND RECOMMENDATION

The results of this study confirm that extracts of different part of *A. paniculata* have antibacterial and antifungal activities. This supports their use in treatment of various infections. The extracts from the different parts of the plants is therefore, recommended at 3000 to 5000 µg/ml concentration for treatment of common bacterial and fungal infections. However, an *in vivo* antimicrobial and toxicological investigations of these extracts are crucial to rationalize its oral administrations in human.

References

[1] Ajayi, I.A., Ajibade, O. and Oderinde, R.A. Preliminary phytochemical analysis of some plant seeds. *Research Journal of Chemical Sciences* 2011;1(3): 58-62.

[2] Ajayi, O.A, Akinola, M. and Awodoyin, R.O. Allelopathic potentials of aqueous extract of *T. diversifolia* in biological control of weeds in cowpea cropping system *International Journal of Agriculture and Economic Development* 2018;5(1): 11-28

[3] Akinpelu, D.A. and Kolawole, D.O. Phytochemical and antimicrobial activity of leaf extract of *Pilliostigma thonningii* (Schum.). *Science Focus Journal* 2007;7: 64-70.

[4] Cheesbrough, M. . District Laboratory Practice in Tropical Countries (Part II). Cambridge University Press, U.K, 2004, 150p.

[5] Kokate, C.K., Purohit, A.P. and Gohale, S.B. Pharmacognosy. Nirali Prakashan Publishers, Pune, India. 2003, 1624.

[6] Kumar, A., Dora, J., Singh, A., Tripathi, R. A review on king of bitter (Kalmegh). *International Journal of Research in Pharmacy and Chemistry* 2012: 2(1):116- 124.

[7] Olajire, A. A. and Azeez, L. Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. *African Journal of Food Science and Technology* 2011; 2 (2): 22-29.

[8] Poongothai, A., Sreena, K.P., Sreejith, K., Uthiralingam, M. and Annapoorani, S. Preliminary phytochemicals screening of *Ficus Racemosa Linn* bark. *International Journal of Pharma and Bio Sciences* 2011; 2(2): 431-434.

[9] Shakil, A.P., Tanushree, S., Sharif, H and Satya, R.S Investigation of the phytochemicals antioxidant, and antimicrobial activities of *Andrographis paniculata* leaf and stem extracts. *Advances in Bioscience and Biotechnology* 2017; 8(5): 149-162.

[10] Suparna, D., Asmita, P. and Punam, S. Study of antioxidants and antimicrobial activities *Andrographis paniculata Asian Journal of Plant Science* 2014; 4(2): 31-41.