

Phytochemical Screening and Antimicrobial Activities of Leaf Extracts of *Andrographis paniculata*

Adegboyega, A. M¹, Oyewole, B. M²

Chemistry Department, The polytechnic, Ibadan

Abstract: Herbs and natural products do not possess much of the toxicity that is present in synthetic chemicals thus enhancing their appeal for long term preventive strategies. The phytochemical and antioxidant potentials of ethanolic, methanolic and aqueous extracts of *andrographis paniculata* (AP) leaves were assessed using standard methods. The antimicrobial activity of Ethanol, Methanol and Water extracts of *andrographis paniculata* (AP) leaves against bacterial strains like *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and fungal strains like *Candida albicans*, *Penicilliumchrysogenum* and *Aspergillus niger* was studied. The antimicrobial activity was determined using the agar well diffusion method. The results revealed the presence of alkaloid, flavonoid, saponin, tannin and the absence of reducing sugars in all the extracts. Antimicrobial analysis showed that ethanolic extract had the highest activity against *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Candida albicans*. The ethanolic leaf extract was observed to possess the highest phenolic and antioxidants content. It can be concluded that AP hold a great promise in the treatment of infections. Our results confer the utility of this plant extracts in developing a novel broad spectrum antimicrobial agent.

Keywords: *Andrographis paniculata*, microorganisms, phytochemicals, agar well diffusion, zone of inhibition

1. Introduction

The world is experiencing an increasing rate of resistance by pathogens to some synthetic drugs hence the challenge to seeking solutions from plant species (Msuya, 1998). Herbs and natural products do not possess much of the toxicity that is present in synthetic chemicals thus enhancing their appeal for long term preventive strategies (Zou, 2005). Though criticized, plant based medicine has survived through ages and it is still catering to the health needs of millions all over the world (Kapoor, 2001). Medicinal plants are considerably useful and economically essential since they contain active constituents called phytochemicals. Phytochemicals are the non nutritive plant chemicals that have protective or disease preventive properties well known examples include phenols, saponins, unsaturated lactones, cytogenic glycosides (Osborn, 1996).

Andrographis paniculata (AP) is a herbaceous plant commonly known as the King of bitters in the family Acanthaceae. AP is also referred to as Bile of the earth due to its bitterness (Coon and Ernst, 2004). It is a plant with characteristic white-purple or spotted purple flowers. The plant grows in waste grounds and prefers moist habitat. AP plant is widely cultivated in Southern Asia where mostly the leaves and roots have been traditionally used as a folklore remedy for a wide spectrum of ailments like diabetics, hypertension, fever, stomach problems and as tonic. Extensive research has revealed that AP has a surprisingly broad range of pharmacologic effects such Anti-inflammatory (Shen et al 2000, 2002), antimalarial (Rahman et al, 1999), Cardiovascular (Tan et al, 2004) and anti-inflammatory activities (Levital et al., 2010; Thiyagarajan et al., 2011). In the present study aqueous, ethanolic and methanolic extracts of AP was investigated for antimicrobial activity.

2. Materials and Method

2.1 Plants Material

Freshleaves of *Andrographis paniculata* (AP) were collected from Abadina College, University of Ibadan, identified and authenticated by Dr. A. Ayodele of Botany Department, University of Ibadan. The leaves were rinsed with tap water and air dried for two weeks, later grinded into fine powder using electric grinder and kept in an air tight container prior to solvents extraction.

2.2 Test Microorganisms

Pure cultures of Bacterial isolates and Fungal Strains were obtained from the University College Hospital (UCH) Ibadan, Nigeria. The obtained Bacterial isolates maintained on NA while the fungal isolates were maintained on PDA.

2.3 Extraction and Concentration

100g of powdered leaves weighed and 400mls of each solvent (Ethanol, Methanol and Water) was added in ratio for 24hrs. The pooled filtrates were then concentrated using Rotary evaporator at the Central Research Laboratory, University of Ibadan. The condensed extracts were then stored in airtight bottles in a refrigerator for further analysis.

2.4 Phytochemical Screening

The AP leaves extracts were screened for various constituents using the method described by Kokate (1993).

2.5 Antimicrobial Assay

The antibacterial and antifungal activities of the extract were studied using the Agar well diffusion method as described by Popoola et al (2007). The bacterial plates were incubated

at 37°C (Fungal plates at 28°C) and the zone of inhibition measured in mm after 24hr of growth. A control experiment was set up by using an equal amount of sterile solvents only in the plates. Each extract was analyzed in triplicate, the mean values are presented.

2.6 DPPH Radical Scavenging Activity

The free radical scavenging ability of the extracts was determined using DPPH assay (Moon and Terao, 1998). Briefly, 0.1 ml of test sample at different concentration (0.1 - 0.9 mg/ml) was mixed with 0.9 ml of Tris-HCl buffer (pH 7.4) and 1 ml of DPPH (500 µM in ethanol). The mixture was shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm in a spectrometer and compared with that of Butylated Hydroxyanisole (BHA). Radical scavenging potential was expressed as IC₅₀ values, which represents the sample concentration at which 50% of the radicals are scavenged. The percentage of DPPH scavenging was calculated using the following formula:

$$\% \text{ Scavenging} = \left[\frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{sample blank}})}{A_{\text{control}}} \right] \times 100$$

2.7 Determination of Total Phenolic Content:

Total soluble phenolic content was estimated by Folin-Ciocalteu method. The extracts were oxidized with Folin Ciocalteu reagent and were neutralized with sodium carbonate. The absorbance of the blue color was measured at 650 nm in a spectrophotometer against a reagent blank. The concentrations of the total phenolic compounds in the extracts were obtained by extrapolating the absorbance of gallic acid on standard gallic acid graph. The concentration of total phenols was expressed as mg/g of dry extract.

2.8 Determination of total phenol

The total phenol content was determined by mixing 0.5 ml of the extract with 2.5 ml 10% Folin-ciocateus reagent (v/v) and 2.0 ml of 7.5% sodium carbonate was added after 3 min. The reaction mixture was incubated at 45°C for 40 min and the absorbance was measured at 765 nm with gallic acid as standard (Singleton et al., 1999).

3. Statistical Analysis

The experimental results are expressed as mean ± standard deviation (SD) of triplicate measurements. The data was subjected to One Way Analysis of Variance (ANOVA)

4. Results

Table 1: Phytochemical constituents of AP leaf extract.

Phytoconstituents	Tests/Reagents	Ethanol	Methanol	Aqueous
Alkaloids	Wagner Reagent	+	+	+
Saponins	Frothing test	+	+	+
Tannins	Ferric Chloride Reagent	+	+	-
Phenolic compounds	Ferric Chloride Reagent	+	+	+
Steroids	Sulphuric acid Reagent	+	+	-
Reducing sugar	Fehlings Reagent	-	-	-
Flavonoids	Acid-alcohol	+	+	-

- = Not detected + = Detected

Table 2: zones of inhibition of different solvent extracts of AP against different pathogens Diameters of zones of inhibition in mm

Microorganisms	Ethanol	Methanol	Water
<i>Salmonella typhi</i>	33.05 ± 3.70	12.25 ± 0.89	4.75 ± 0.01
<i>Shigella dysenteriae</i>	33.05 ± 2.26	15.75 ± 0.02	7.25 ± 1.70
<i>Staphylococcus aureus</i>	42.00 ± 3.39	5.25 ± 0.01	6.75 ± 3.05
<i>Candida albicans</i>	24.05 ± 1.70	10.05 ± 1.45	1.75 ± 0.02
<i>Penicillium travenetum</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Aspergillus niger</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are means of three independent replicates.

Table 3: Phenol and Antioxidants content of *Andrographis paniculata* leaves in different solvents

Sample	Phenols (GAE/100g)	DPPH (% inhibition)
Aqueous extract	45.9 ± 0.75	16.0 ± 0.20
Ethanol extract	49.5 ± 0.70	17.8 ± 0.33
Methanol extract	48.7 ± 0.33	15.5 ± 0.01

5. Discussion

Medicinal plants are nature's gift to human beings to lead a disease-free and healthy life. Antibiotic resistance has become a global concern hence the continuous and urgent need to discover new antimicrobial compounds with diverse chemical constituents and new mechanism of action. The phytochemical screening showed that the different solvent extracts of AP revealed the presence of tannin, flavonoids, phenol, alkaloids, steroids, anthraquinones and saponins. The phytochemicals strongly present in the ethanol and methanol extracts. But the water extract yielded less quantity of phytochemicals (data is not presented). Hence the presence of these constituents shows the importance of AP which could be used to cure various ailments as observed in Table 1. The presence of compounds like phenols, tannins, flavonoids and alkaloids in the extracts might be responsible for the antimicrobial activity. However reducing sugar was observed to be absent in all the extracts. The antibacterial activity of different solvent extracts against the pathogenic bacteria showed varied levels of inhibition as presented in table 2.

Results obtained in the current investigation revealed that the extracts possess potential antibacterial activity against entire tested organisms, however ethanol extract was found to have shown the strongest and broadest spectrum. Ethanol extract was most effective as it exhibited the maximum significant inhibition against *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Candida albicans*. Among the solvent extracts tested, ethanol extract had a broad spectrum of activity and showed the highest zone of inhibition against *S. aureus* (42.00 ± 3.39 mm). This may be due to the presence of tannins. Tannins are known for their astringent property and antimicrobial activity (Cowan, 1999). The least zone of inhibition for all the tested bacteria was observed with the water extract. The results obtained in the present study indicate that the ethanol extract is more active against the pathogenic bacteria and has a broad spectrum activity.

Also in the present study, we observed that ethanol leaf extract had the highest phenolic and antioxidants content hence antibacterial activity of ethanol extract can be said to be due to the presence of these compounds (Kathad et al, 2010). Therefore variation in the antimicrobial activity of different solvents can be rationalized in terms of the polarity of the solvents used, polarity of the compounds being extracted by each solvent and, in addition to their extrinsic bioactivity and by their ability to dissolve or diffuse in the media used in the assay (Anjana et al, 2009). These results confirmed the substantiation of previous studies which have reported that organic solvent is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as water (Emad et al, 2009)

6. Conclusion

The present study showed interesting results based on the bacteriocidal and fungicidal activities of the AP plant. Antibacterial activity obtained varied with solvents used for extraction. It can be concluded that crude ethanolic extract of AP plant has a promising medicinal value and treatment for the alleviation of symptoms of infections caused by microorganisms like *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysenteriae* and *Candida albicans*. Also ethanolic extract of *A. paniculata* leaves showed DPPH scavenging activity and phenolic content. Plant phenolic compounds have been found to possess potent anti-inflammatory activity (Sakat et al., 2010). On the basis of the results obtained, it can be concluded that ethanol is the best solvent for extracting antimicrobial and antioxidant bioactive compounds. Hence AP plant is worthy of consideration though further phytochemical work need to be done on these extracts including fractionating to isolate and elucidate the structure of the bioactive compounds.

7. Recommendation

The identification and isolation of the active compounds could lead to the discovery of cheaper phytomedicines and to overcome resistance developed by microorganisms due to dosage incompleteness. Research into the benefits of medicinal plants needs to be encouraged because appreciable number of people benefits from medicinal plants. The use of plant

extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported various types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metal. As a result, sterilization is needed especially for aqueous extracts before use to get rid of these contaminations

References

- [1] Anjana S, Rani V and Padmini R. Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens. *World Applied Science Journal* 2009; 7 (3): 332-339.
- [2] Coon J. T and Ernst E. T (2004): *Andrographis paniculata* the treatment of upper respiratory tract infections: Assystemic review of safety and efficacy. *Plantamadica*. 70 (4):293-298.
- [3] Kapoor L. D (2001): *Handbook of Ayurvedic medicinal plants*. CrcPress Florida 416.
- [4] Kathad HK, Shah RM, Sheth NR and Patel KN. In vitro antioxidant activity of leaves of *Garugapinnata* Roxb. *International Journal of Pharmaceutical Research* 2010; 2 (3): 9- 13.
- [5] Kokate C. K (1993): *Practical Pharmacognosy*, (4Ed) Vallabh Prakashan pp107-111, 178-181.
- [6] Moon JH and Terao J. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low density protein. *Journal of Agricultural and Food Chemistry* 1998; 46: 5062-5065.
- [7] Levita, J., A. Nawawi, A. Mutalib and S. Ibrahim. (2010): Andrographolide: A review of its anti-inflammatory activity via inhibition of NF-kappaB activation from computational chemistry aspects. *International Journal of Pharmacology*, 6:569-576.
- [8] Osborun A. E. (1996): Performed Antimicrobial Compounds and Plant Defense against Fungal attack. *The Plant cell* 8: 1821-1831.
- [9] Popoola TOS, Yangomodu O. D., Akintokun A. K. (2007): Antimicrobial Activity of Cassava Seed Oil an Skin Pathogenic Microorganisms Res. *J. Medicinal Plant* 1 (2): 60-64
- [10] Rahman, NNN, Furuta A, Kojima T, Tabane S and Ali-Mohd M (1999): Anti-malarial activity of extracts of Malaysian medicinal plants *J. Ethnopharmacol*. 64; 249-254.
- [11] Sakat S, Juvekar AR, Gambhire MN., In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharma and Pharmacological Sciences*. 2 (1): 146-155, (2010).
- [12] Shen Y. C, Chen C. F and Chion W. F (2000): Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide. *Plant med* 66:314-317
- [13] Shen Y. C, Chen C. F and Chion W. F. (2002): Andrographolide prevents oxygen radical production by human neutrophils, Possible mechanism (s) involved in its anti-inflammatory effect. *Br. J. Pharmacol*. 135; 399-406.
- [14] Thiyagarajan P, Deepak HB and Agarwal A (2011): In vitro modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of *Andrographis paniculata* (King of bitters) extract. *International Immunopharmacology*. 11:79-84.
- [15] WHO. ICU and WWF (1993): Guidelines on the Conservation of medicinal plants, IUCNG land, Switzerland, 1;4-6
- [16] Zou D., Brewer M., Garcia F., Feugan J. M., Wang J., Zang R., Liu H. and Zou C. (2005): Cactus Pear: A Natural Product in Cancer Chemoprevention. *Nutritional Journal* 4-25.