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Antimicrobial properties and medicinal effects of *Andrographis paniculata*

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Abstract

Antimicrobial properties and medicinal effects of *Andrographis paniculata* was carried out in this study. *Andrographis paniculata* leaves were collected from Federal Polytechnic Nekede, Owerri and identified by a botanist. The clinical isolates of *Klebsiella specie*, *Vibrio cholera* and *Escherichia coli* were obtained from the Microbiology Department of Federal Medical Centre, Owerri, identified and isolates were then sub-cultured on sterile Nutrient agar and the microbial culture were diluted with peptone water until the final suspension that contained about 1.5 x 10^5cfu/ml of *Vibrio cholera* and *Escherichia coli* and 1.0 x 10^5cfu/ml of *Klebsiella specie*, were obtained according to the method of Akujobi *et al.*, (2004). Hot water and alcoholic extraction were adopted for this study. Results of the ethanolic extracts of *Andrographis paniculata* against the enteric bacteria showed that the ethanolic extracts had zones of inhibition of 10mm and 19 mm against *Vibrio cholera* and *Klebsiella species*. The minimum bactericidal concentration of the *Andrographis paniculata* against the enteric bacteria were 100 mg/ml each against *Klebsiella species* and *Vibrio cholera*. The minimum bactericidal concentrations were 200 mg/ml each against *Klebsiella species* and *Vibrio cholera*. This work has therefore shown that the ethanolic extracts of *Andrographis paniculata* can be used in treatment of diseases caused by susceptible enteric bacteria.

Keywords: *Andrographis paniculata*, antimicrobial, bactericidal, phytochemical

1. Introduction

1.1 Background to the study

A great number of people around the world in most countries depend highly on herbs to obtain some health benefits. Use of conventional drugs have proved to cause various health problems. These problems have made researchers revalue the therapeutic potentials of many types of plants with medicinal properties depending on their variation. There is an urgent need to examine past literature gotten on some herbs in order to add to the existing data which is vital. One of them is *Andrographis paniculata* (Acanthaceae). It is made up of about 40 species which has served as an ayurveda since ancient times. Characterised by its Bitters taste, It also grows yearly (Ghosh *et al.*, 2012) [2]. It can be found in India, Srilanka and also spreads to various parts of Southeast Asia and America. It thrives in a variety of soil which allows it grow in different places. *Andrographis paniculata* is grown as a result of its famous medicinal benefits. Roots of *A. paniculata* have proved to be an effective form of treating different ailments. Numerous research have been performed by various scientist and reports about medicinal potentials contained in this herb have been made. Phytochemicals contained in *A. paniculata* includes diterpenoid lactones, flavonoids and other miscellaneous compounds. These phytochemicals in *A. paniculata* helps it possess numerous pharmacological properties (Lim *et al.*, 2012) [4].

Andrographis paniculata (AP) commonly known as *Kalmegha* in Hindi, *Kalamegha* in Sanskrit and *Kalmegh* in Bengali is an erect herb belonging to family Acanthaceae which grows in many south east Asian countries and in India. The plant is highly regarded for its therapeutic potential in Indian phytotherapy and traditional medicine. Both crude and alcoholic extracts of AP has shown different pharmacological activities *viz*, antibacterial, antifungal, antiviral, anthelmintic, anticancer, hyperglycemic, anti-inflammatory, antivenomic, in alleviation of upper respiratory tract infections, hepatoprotective, preventive effects against cold. The herb grows upto 3-4 feet in height, the leaves are lanceolate and 2-3 inches long (Lee *et al.*, 2010) [3].

The flowers are small, solitary and flowering time is from September to December. Traditionally the leaves of this herb are used for bronchitis, worm infestation, influenza and dyspepsia. The expressed juice of the leaves is a domestic remedy in flatulence and diarrhoea. There is need for critical evaluation since few scientists have reported side effects of *Andrographis paniculata* (Wong *et al.*, 2016) [7]. Therefore, this study assess the antimicrobial properties and medicinal effects of *Andrographis paniculata*.

2. Materials and Methods

2.1 Sample collection and Processing

Andrographis paniculata leaves were collected from Federal Polytechnic Nekede, Owerri and identified by a botanist. The samples were air-dried and grinded into powder using sterile manual grinder (sterilized with 90% ethanol). This was kept in an air-tight glass containers

protected from light and heat until required for further studies.

2.2 Preparation of test organisms

Isolates of *Klebsiella specie, Vibrio cholera* and *Escherichia coli* were procured from the Microbiology Department of Federal Medical Centre, Owerri, identified and then sub-cultured on a sterile Nutrient agar. The microbial culture were diluted with peptone water until the final suspension that contained about 1.5 x 10^5cfu/ml of *Vibrio cholera*, *Escherichia coli* and 1.0 x 10^5cfu/ml of *Klebsiella specie*, were obtained according to the method of Akujobi *et al*., (2004) [1]. The cell densities obtained were in accordance with 0.5 McFarland's standard and used in all the investigations. The McFarland's standard was prepared by adding 0.1ml of 1% $BaCl_2$ into 9.9ml of 1% Sulphuric acid.

2.3 Extraction of the plant materials

Hot water extraction and alcoholic extraction with ethanol (99%) as described in Association and Analytical Chemists AOAC (1990) was used for this study. Twenty grams (20 g) of the grounded sample of the cashew leaf was weighed into about 100 ml of water and heated. It was stirred intermittently for 30 minutes and allowed to boil. After boiling, it was filtered using Whatmann's filter paper. For the cold water extraction, the (20 g) of the ground samples were placed in 100 ml of distilled cold water and allowed to stay for 4 hours before it was filtered us Whatmann filter paper. For the ethanolic extraction, 20 g of the ground sample was stuffed in a thimble and placed in the extraction chamber of the soxhlet extractor. Thereafter the Liebig condenser containing the water in-let and outlet hoses was fitted into the extraction chamber which was then placed into a flat bottom flask containing 200 ml of ethanol. The apparatus was set up on a heating mantle and then the mantle was connected to the mains. After the extraction, the ethanol was recovered from the mixture of ethanol and the extract using simple evaporation techniques. The Soxhlet extract was used in all the investigations.

2.4 Antimicrobial susceptibility testing of the plant extracts

The disc technique as demonstrated by Osadebe and Ukwueze (2004) [5] was used for this study to evaluate the antibacterial activity of the extracts. 0.2ml aliquot of each of the extract was dropped on sterile filter paper discs of 6 millimetres in diameter and allowed to get absorbed before they were placed into Nutrient agar plates and sabouraud dextrose agar inoculated with each of the test organisms and appropriately labeled. Discs impregnated with water and ethanol were used as control in each case. The nutrient agar plates and sabouraud dextrose agar were then incubated at 37 °C for 24 hours and 25 °C for 48 hours. The zones of inhibitions were measured with a meter rule.

2.5 Test for minimum inhibitory concentrations (mic) of the plant extracts.

For the MIC test, the plant extract was concentrated by evaporation and one gram (1g) of the extract was dissolved in four millilitre (4 ml) of peptone water; this gives 250 mg/ml. Also, 0.8 g of the same extract was placed in 4 ml of peptone water to obtain the concentration of 200 mg/ml. Thereafter, two fold serial dilutions was carried out from the 200 mg/ml concentration by transferring 2 ml of the 200 mg/ml concentration to 2 ml of peptone water contained in a test tube and homogenized properly. This procedure of transferring 2 ml of the tube to 2 ml of peptone water contained in the subsequent tubes was continued until the fifth tube. The following concentrations were thereafter obtained: 250 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml. Having obtained the different concentrations and dilutions, three drops of overnight broth cultures of the test organisms were inoculated into the dilutions in each case of the test organisms (Akujobi *et al*., 2004) [1]. The tubes were then incubated at 37 °C for 24 hours. The lowest concentration of each of the extracts in each case of the extracts (hot, cold and alcoholic extracts) that inhibited the growth of the test organisms were recorded as the MIC.

2.6 Test for the bactericidal/fungicidal concentrartion of the extract

Tubes showing no visible growth from the MIC test were sub cultured onto sterile Nutrient agar plates and incubated at 37 °C for 24 hours. The lowest concentration of the extracts that showed growth was recorded as the Minimum Bactericidal/Fungicidal Concentration as the case may be.

3. Results and Discussion

3.1 Results

Antibacterial susceptibility test of the extracts of *Andrographis paniculata* is presented in table 1, table 2 contains the results of the Minimum Inhibitory Concentration of the extracts and table 3 contains the result of the a Minimum bactericidal concentration of the extracts.

Table 1: Antimicrobial susceptibility testing of the extracts

Test organism	Ethanolic extract (mm)	Aqueous extract (mm)	Control Chloramphenicol/Nystatin (mm)
Escherichia coli	-	-	20
Vibrio cholera	10	-	10
Klebsiella species	19	-	18

Key: -=No Zone, CHL =Chloramphenicol, NYS = Nystatin

Table 2: Minimum Inhibitory Concentration (MIC) of the plant extracts

Test organism	Ethanolic extract (mg/ml)	Aqueous extract (mg/ml)
Escherichia coli	ND	ND
Vibrio cholera	100	ND
Klebsiella species	100	ND

Key: N.D = Not determined because the raw extract did not show any zone of inhibition.

Table 3: Minimum Bacterial Concentration (MBC) of the plant extracts

Test organism	Ethanolic extract (mg/ml)	Aqueous extract (mg/ml)
Escherichia coli	ND	ND
Vibrio cholera	200	ND
Klebsiella species	200	ND

Key: N.D = Not determined because the raw extract did not show any zone of inhibition.

4. Discussion and Conclusion

The results of the antimicrobial properties of the ethanolic extracts of *Andrographis paniculata* against enteric bacteria as presented in Table 1 showed that the ethanolic extracts had zones of inhibition of 10 mm and 19 mm against *Vibrio cholera* and *Klebsiella species*. The minimum bactericidal concentration of the *Andrographis paniculata* against the enteric bacteria were 100 mg/ml each against *Klebsiella species* and *Vibrio cholera*. The minimum bactericidal concentrations were 200 mg/ml each against *Klebsiella species* and *Vibrio cholera*. Punitha *et al.*, (2019) [8] also reported the antimicrobial properties of *Andrographis paniculata.* In their reports, *Escherichia coli*, *Streptococcus faecalis* and *Staphylococcus aureus* were susceptible to the extracts. This is in line with the outcome of this study. The ethanolic extracts exhibited higher antimicrobial activities compared with the aqueous extract. The significant antimicrobial activities of ethanolic extract of *Andrographis paniculata* may be due to varying degree of solubility of the active constituents in the solvents. Rajendrakumar *et al.*, (2009) [9] also reported the presence of alkaloids and phenolic compounds in the extracts. Similar findings were gotten in this study. The antimicrobial properties of *Andrographis paniculata* may be attributed to the presence of these phytochemicals.

5. Recommendation

Obtained results in this study has necessitated the need for Government to support research in pharmaceutical microbiology to determine plants with antimicrobial properties. Plant species should be further explored for antimicrobial activities thereby finding solution to the growing cases of drug resistance. The extracts of *Andrographis paniculata* should further be purified and used in the production of drugs.

6. Conflict of interest

The authors have declared no conflict of interest.

7. References

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