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ANTIBACTERIAL SCREENING OF *ANDROGRAPHIS ECHIOIDES* (L.) NEES AGAINST SELECTED HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Andrographis echioides (L.) Nees is a valuable medicinal plant. The present study is to investigate the antibacterial activity of the various leaves and stem extracts of *A. echioides.* Shade dried plant leaf and stem of *A. echioides* were tested for antibacterial the antibacterial activity of different extracts (petroleum ether, chloroform, acetone, methanol and ethanol) of leaves and stem of *A. echioides* using the standard disc diffusion assay against four gram-positive bacterial species, viz., *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Staphylococcus aureus* and *Bacillus cereus* six gram-negative bacterial species viz., *Escherichia coli*, *Serratia marcescens*, *Enterobacter amnigenus, Klebsiella pneumonia, Klebsiella oxytoca* and *Brevibacterium paucivorans.* Among various solvent extracts studied acetone, methanol and ethanol leaf and stem extract showed a highest antibacterial assay followed by chloroform and petroleum ether. No results found in petroleum ether leaf and stem extract against all test bacteria. Hence the present study justifies the claimed uses of this herb in the traditional system of medication to treat various sicknesses. This report is an antibacterial screening from *A. echioides* leaf and stem.

KEYWORDS: *Andrographis echioides*, Antibacterial, Disc diffusion method.

INTRODUCTION

Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability.[1] From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products.[1] In the last few decades, many bacterial organisms have continued to show increasing resistance against current antimicrobial agents.[2] Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make

them a rich source of many potent drugs.[3] The use of herbal medicinal plants has always played a positive role in the control or prevention of diseases such as diabetes, heart disorders and various cancers.[4] Some medicinal plants have been used in the production of various drugs singly or in combination and even as principal raw material for the production of other conventional medicines.[5] The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal remedies. In this connection, plants continue to be a rich source of therapeutic agents. The active principles of many drugs found in plants are produced as secondary metabolites.[6]

Andrographis echioides (L.) Nees (Gopuram thanki) is one of the important medicinal plant species belonging to the Acanthaceae family. The genus consists of 40 species distributed in Tropical Asia.[7] The whole plant extract is applied topically over fungal infections, to control hair fall, snake bite, cuts and wounds.[8] In Indian system of medicine like Ayurvedha, Siddha and Unani have been in existence for over several countries. This traditional system of medicine has served 70% of people of developing countries, for the variety of diseases.[9] Medicinal properties of this plant are more or less similar to those of *Andrographis paniculata* a widely studied plant for wide range of pharmacological activities.[10] Hence the present investigation was carried out to analyze the antibacterial activity against selected bacterial pathogens from *Andrographis echioides*.

MATERIAL AND METHODS

Plant Material

The aerial part of plant of *Andrographis echioides* (L.) Nees was collected from Thuraiyur region of Trichy district

Preparation of Plant Powder

The plant parts were carefully examined and old insect damaged, fungus infected leaf and stem were removed. The selected healthy plant parts were spread out and shade dried in the laboratory at room temperature for 5-8 days or until they broke easily by hand. The dried plant parts were ground to a fine powder by using an electronic blender and the powders were stored in a closed container at room temperature for further uses.

Plant Extraction

Solvent Extracts

Fifty grams of the powdered plant material (leaves and stem) was boiled separately with 300 ml of each of the solvents viz. petroleum ether, chloroform, acetone, methanol and ethanol in a soxhlet apparatus for 48 h at different temperatures (depends on the boiling point of the respective solvents). At the end of 48 h each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature. The paste like extracts were stored in pre-weighed screw cap bottles and the yield of extracts was calculated based on initial and final weight of the container. These screw cap bottles with the extracts were kept in refrigerator at 4℃. Each of the extract was individually reconstituted by using minimal amount of the extracting solvent prior to use.

Antibacterial Activity Test (Disc diffusion method)[11]

Disc Preparation

The filter paper discs of uniform size are impregnated with the compound (plant extract) usually consisting of absorbent paper. It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dried discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These dried discs were used for the test.

Test Bacteria

Antibacterial activity of *Andrographis echioides*(L.) Nees powder extracts was investigated against ten bacterial species such as *Staphylococcus haemolyticus, Staphylococcus lentus, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Serratia marcescens, Enterobacter amnigenous, Klebsiella pneumoniae, Klebsiella oxytoca and Brevibacterium paucivorans.* These species were purchased from Department of Microbiology, K.AP Viswanatham medical college, Tiruchirappalli, Tamil Nadu.

Method

Sterile Nutrient Agar medium (pH 7.4 ± 2) was poured (10-15 ml) into each sterile petriplates. The growth media also seem to play an important role in the determination of the antibacterial activity. After solidification, 100 µl of suspension containing 108 CFU/ml of each test bacteria was spread over Nutrient Agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the 3 mg/ml extracts (30µg/disc) placed on the inoculated agar. Negative controls were prepared in using the same solvents employed to

dissolve the plant extract. Chloramphenicol (30µg/disc) was used as positive reference control to determine the sensitivity of the plant extract on each bacterial species. The inoculated plates were incubated at 37° C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones. Each assay was conducted in triplicate.

Statistical analysis

Agar disc diffusion activity was performed in three replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analyzed and expressed as Mean ± Standard Deviation.

RESULT AND DISCUSSION

The *in vitro* antibacterial activity of the different solvent extracts of *Andrographis echioides* was evaluated by disc diffusion assay against clinical pathogenic bacteria. The determination from the present study showed that the five leaf and stem extracts (petroleum ether, chloroform, acetone, methanol and ethanol) of *Andrographis echioides* revealed antibacterial properties against all the five human pathogens tested. All the extracts exhibited significant activity except petroleum ether. Among 5 solvent extracts, acetone and ethanol showed broad spectrum of activity against the tested bacteria. The acetone leaf extract showed greater inhibition against *Staphylococcus lentus* (18.3+3.7), *Bacillus cereus* (18+1.5), *Staphylococcus haemolyticus* (14.3+1.5), *Klebsiella oxytoca* (11.3+3.0) and *Staphylococcus aureus* (10.6+1.5). Moderate inhibition zones were observed against *Serratia marcescens* (6.3+0.5). Lowest inhibition was observed against *Enterobacter amnigenus* (4.6+4.1). No inhibitory activity was observed against *Escherichia coli* and *Klebsiella pneumonia*. The ethanol extract also exhibited better activity against all the tested bacteria. In ethanol extract, highest inhibition zones were observed against *Bacillus cereus* (14.3+1.5), *Brevibacterium paucivorans* (14+1.7), *Staphylococcus haemolyticus* (11.6+0.5) and *Staphylococcus lentus* (11.6+4.0). Moderate inhibition was observed against *Escherichia coli* (9+1.4), *Enterobacter amnigenus* (8.3+1.3), *Klebsiella oxytoca* (8+1), *Staphylococcus aureus* (7.6+0.6) and *Serratia marcescens* (7.3 + 0.5).

Least inhibition was observed against *Klebsiella pneumonia* (6.3+0.5). The extract obtained using methanol showed a highest activity against *Klebsiella oxytoca* (12+1), *Bacillus cereus* (12+3), *Serratia marcescens* (11.6+0.5), *Staphylococcus lentus* (11.3+1.1) and *Enterobacter amnigenus* (11+3.6). Moderate inhibition was observed against *Staphylococcus haemolyticus* (10+1) *Brevibacterium paucivorans* (9.3+2.3) and *Staphylococcus aureus* (8.6+1.5). Least

inhibition zone was observed against *Escherichia coli* (7+1). The chloroform extract also showed inhibition against some of the tested bacteria pathogens.

The methanolic stem extract showed significant activity against all the tested bacteria pathogen. The highest inhibitory zones were observed against *Staphylococcus lentus* (16.6+1.5), *Bacillus cereus* (12.6+2.0), *Serratia marcescens* (11.6+1.5) and *Brevibacterium paucivorans* (11.3+2.0) and *Staphylococcus haemolyticus* (11+7). Minimum activity observed against *Staphylococcus aureus* and *Klebsiella oxytoca* (10.6+0.5), *Klebsiella pneumonia* (9.6+2.5), *Enterobacter amnigenus* (8+1.7). There is no least inhibition against bacteria pathogens. Similarly ethanolic extract also possess significant activity against all tested pathogens followed by acetone and chloroform. The ethanol stem extract showed the diameter of inhibition zones ranging from 6.0 to 13mm. With the highest inhibition zone observed against *Bacillus cereus* (13.3+0.5). Minimal inhibition zone was noticed against *Enterobacter amnigenus* (6.3+0.5mm). Observation made from chloroform extract showed a highest activity against *Bacillus cereus* (17+1mm) and the minimum activity against *Staphylococcus lentus* (11+1.7mm), *Klebsiella oxytoca* (9.3+1.1) and *Enterobacter amnigenus* (9+1.1), where it has no antibacterial against *Staphylococcus aureus*, *Serratia marcescens*, *Enterobacter amnigenus*. The extract obtained using chloroform showed a highest activity against *Klebsiella pneumonia* (10.3+3.2), *Brevibacterium paucivorans* (10+1.7). Where it has no activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter amnigenus* and *Klebsiella oxytoca* in both the leaf and stem extract petroleum ether solvent extract showed no activity against all the tested bacteria.

The diameter of inhibition zones for each of the samples were compared with standard. It was noted that the inhibition. Zones of the equal to the inhibition zones of standard antibiotics he leaf extracts exhibited high degree of inhibition than the other parts used.

In our study, the acetone leaf extract and methanolic stem extract of *Andrograpis echioides* has shown various degrees of inhibitory activity on all the bacterial strains used. The plant extract as found to have a highest inhibitory activity against gram positive bacteria. Generally gram positive bacteria were more sensitive to plant extract because of the presence of mesh like peptidoglycan layer which is more accessible to permeation by the extracts.[12and13] The resistance of the gram negative could be attributed to its cell wall structure. Gram negative bacteria have a powerful permeability barrier, composed of a thin lipopolyaccharide exterior membrane, which could restrict the penetration of the extruding plant extract.[13and14]

Table – 1: Antibacterial screening of leaf extracts of *Andrographis echoides* (L.) Nees on pathogenic bacteria (Disc diffusion method)

Test bacteria	Inhibition zone diameter in mm (mean ± SD)										
	Petroleum ether		Chloroform		Acetone		Methanol		Ethanol		Positive Control
	Experiment (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimen (30 µg/disc)	Negative control	Chloramphenicol (30 mcg/disc)
Gram- Positive											
Staphylococcus hemilyticus	-	-	-	-	14.3±1.5	-	10.3±1.1	-	11.6±0.5	-	24.3±1.1
Staphylococcus lentus	-	-	8±0	-	18.3±3.7	-	11.3±1.1	-	11.6±4.0	-	23±0
Staphylococcus aureus	-	-	-	-	10.6±1.5	-	8.6±1.5	-	7.6±0.6	-	26.6±0.5
Bacillus cereus	-	-	-	-	18±2	-	12±3	-	14.3±1.5	-	18.6±0.5
Gram-negative											
Escherichia coli	-	-	4.6±4.0	-	-	-	7±1	-	9±1.4	-	25±0
Serratia marcescens	-	-	4.3±3.7	-	8.6±0.5	-	11.6±0.5	-	7.3±0.5	-	28±0
Entrobacter aerogens	-	-	-	-	4.6±4.1	-	11±3.6	-	8.3±1.3	-	24±1
Klebsiella pnemoniae	-	-	4±3.4	-	-	-	10±1	-	6.3±0.5	-	12.3±0.5
Klebsiella oxytoca	-	-	4.6±4.0	-	11.3±3.0	-	12±1	-	8±1	-	15.3±2.5
Brevibacterum paucivorans	-	-	-	-	6.3±0.5	-	9.3±2.3	-	14±1.7	-	30±0

'–' represents as 'no inhibition'

Table – 2: Antibacterial screening of stem of extracts of *Andrographis echoides* (L.) Nees on pathogenic bacteria (Disc diffusion method).

Test bacteria	Inhibition zone diameter in mm (mean ± SD)										
	Petroleum ether		Chloroform		Acetone		Methanol		Ethanol		Positive Control
	Experiment (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimen (30 µg/disc)	Negative control	Chloramphenicol (30 mcg/disc)
Gram- Positive											
Staphylococcus hemilyticus	-	-	9.3±1.1	-	8.6±2.0	-	11±1.7	-	12±1	-	24.3±1.1
Staphylococcus lentus	-	-	8.6±1.1	-	11±1.7	-	16.6±1.5	-	10.3±1.5	-	23±0
Staphylococcus aureus	-	-	-	-	-	-	10.6±0.5	-	8±1	-	26.6±0.5
Bacillus cereus	-	-	6.6±0.5	-	17±1	-	12.6±2.0	-	13.3±0.5	-	18.6±0.5
Gram-negative											
Escherichia coli	-	-	-	-	8.6±2.0	-	10.6±2.0	-	10±4.5	-	25±0
Serratia marcescens	-	-	7.6±0.5	-	-	-	11.6±1.5	-	9.3±0.5	-	28±0
Entrobacter aerogens	-	-	-	-	-	-	8±1.7	-	6.3±0.5	-	24±1
Klebsiella pnemoniae	-	-	10.3±3.2	-	8.3±1.5	-	9.6±2.5	-	10±2.6	-	12.3±0.5
Klebsiella oxytoca	-	-	-	-	9.3±1.1	-	10.6±0.5	-	8.6±1.5	-	15.3±2.5
Brevibacterum paucivorans	-	-	10±1.7	-	9±1.7	-	11.3±2.0	-	10.6±2.3	-	30±0

'–' represents as 'no inhibition'

The present study results similar with the results of[15] He reported that the acetone and alcohol extracts of *Andrographis paniculata* with higher inhibitory activity *Bacillus cereus* and *Staphylococcus aureus*. In this study methanolic stem extract of *A. echioides* exhibited better activity against all tested pathogens. This result was supported by the results of[16] Have reported that the methanol extract of *Andrographis paniculata* leaves was found to be active against *Staphylococcus aureus, Enterocccus faccalis* This results are in accordance with the previous study reported that the methanol extract of *Andrographis paniculata* showed antibacterial activity against *E.coli*[17] had also reported that the hexane, chloroform and methanol extract of *Andrographis paniculata* showed antibacterial activity again *E.coli* through disc diffusion method.

CONCLUSION

All the solvents extracts of *A. echioides* extracts except petroleum ether exhibited varying degree of inhibition activity against the growth of the entire tested microorganism. The inhibition against both gram-positive and gram-negative bacteria. Acetone, methanol and ethanol extract of *Andrographis echioides* is worthy of further investigation as a natural antibacterial agent and evaluate the other parameters of antimicrobial activity.

REFERENCE

1. Shahzadi, I., Hassan, A., Khan, U.W., and Shah, M.M. Evaluating biological activities of the seed extracts from Tagetes minuta L. found in Northern Pakistan. Journal of Medicinal Plants Research, 2010; 4(20): 2108-2112.
2. Nascimento, G.G., Locatelli, J., Freitas, P.C., and Silva, G.L. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, Brazilian Journal of Microbiology, 2000; 31(4): 247-256.
3. Srivastava, J., Lambert, J., and Vietmeyer, N. Medicinal plants: An expanding role in from Western India for potential antimicrobial activity. Indian Journal of Pharmacology, 2005; 37: 406 - 409.
4. Mohanta, B., Chakraborty, A., Sudarshan, M., Dutta, R., and Baruah, M. Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. Journal of Radioanalytical and Nuclear Chemistry, 2003; 258(1): 175-179.
5. Tahir, L., and Khan, N. Antibacterial potential of crude leaf, fruit and flower extracts of Tagetes Minuta L. Journal of Public Health and Biological Sciences, 2012; 1: 70-74.

6. Kianbakht S, Jahaniani F, "Evaluation of Antibacterial Activity of Tribulus terrestris L. Growing in Iran," Iranian Journal of Pharmacology and Therapeutics, 2003; 2(1): 22-24.
7. Anonymous, "Wealth of India-Raw Materials", Vol.1, CSIR Publictions Co., New Delhi, 1948; 76-87.
8. Anonymous. Andrographis echioides(L.) Nees in Wall., Pl. Asiat. Rar. 3: 117. 1832; Hook. f., Fl. Brit. India 4: 505. 1884; Gamble, Fl. Pres. Madras 1051(736). 1924; Vajr., Fl. Palghat Dist., 1990; 341.
9. Gangoue-pieboji J, Pegnyemb DE, Niyitegeka D. The in-Vitro Antimicrobial Activities Ghosh et al. World Journal of Pharmacy and Pharmaceutical Sciences of Some Medicinal Plants from Cameroon. Annals of Trop Medicinal and Parasitology, 2006; 100(3): 273-243.
10. Kokate CK. Practical Pharmacognosy, Vallabh Prakshan, New Delhi, 1994; 107-113.
11. Bauer AW, Kirby WM, Sherri's JC, Truck M. Antibiotic susceptibility testing by a Standardized single disk method. Am. J. clin. Patol, 1966; 45(4): 493-496.
12. Rameshkumar KB, George V, Shiburai. Chemical constituents and antibacterial activity of the leaf oil of Cinnamomum chemungianum Mohan et Henry. Journal of Essential Oil Research, 2007; 119(1): 98-100.
13. Tajkarimi M M, Ibrahim S A, Cliver D O, Antimicrobial herbal and spice compound in food, Food control, 2010; 21(9): 1199-1218.
14. Stefanello MEA, Cervi AC, Ito YI, Salvador MJ, Wisniewski A, Simionatto EL. Chemical composition and antimicrobial activity of essential oils of Eugenia chlorophylla (Myrtaceae).Journal of Essential Oil Research, 2008; 20(1): 75-78.
15. Hosamani PA, Lakshman HC, Kumar SK, Rashmi C, Hosamani. Antimicrobial activity of leaf extract of Andrographis paniculata wall. Science Researh Reporter, 2011: 1(2): 92-95.
16. Mishra PK, Rahoul Kunwar S, Anamika G, Adya C, Rahul P, Shree Prakash T et al. Antimicrobial activity of Andrgraphis paniculata (Burm.f) wal ex Nees leaves against clinical pathogens Journal of Pharmacy Research, 2013: 7: 457-462.
17. Premanath and N. Devi., Antibacterial, Antifungal and Antioxidant Activities of Andrographis Paniculata Nees. Leaves, International Journal of Pharmaceutical Sciences and Research, 2011; 2091-2099.