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Antibacterial activity of *Andrographis paniculata* Nees against selective human pathogens

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Abstract – The present study was conducted to investigate the antibacterial effect of Andrographis paniculata against selective human pathogens. The plant parts of A. paniculata such as leaf, stem and root were studied for its antibacterial activity. Different solvents were used to extract the active components from the plant parts. The antibacterial activity was studied against selective human pathogens viz., Staphylococcus sp., Escherichia coli, Salmonella sp. and Pseudomonas sp. Among the different solvents, methanol extract showed greater antibacterial activity against E. coli (32.8 mm) followed by Salmonella Typhi (24.7 mm), Pseudomonas sp. (24.2 mm) and Staphylococcus sp. (18.4 mm). So the future investigation was carried out for leaf extract using methanol as solvent to find out the minimum inhibitory concentration for selective human pathogens. The results reveal that 75 µl was optimum for all the test cultures. It shows more activity in E. coli (32.3 mm) followed by S. Typhi (28.1 mm), Staphylococcus sp. (14.1 mm) and Pseudomonas sp. (13.4 mm).

Keywords -Andrographis paniculata, Antibacterial, Andrographolide, minimum inhibitory concentration.

I. INTRODUCTION

Medicinal plants have grown enormously from the use of herbal products as natural cosmetics and as self-medication by the general public scientific for their beneficial effects (Sharma and Joshi, 2011). In olden ages, antibiotics were produced mostly from the leaves and roots of medicinal plants. The extracts of many plant species have become popular in recent years and attempts have taken to characterize their bioactive principles which gained scope for various pharmaceutical food processing and medical application. *Andrographis paniculata* is an herbaceous plant of the family *Acenthaceae*, native to India and Srilanka. In North-Eastern India the plant is known as Maha-tita literally “King of bitters”, known as various vermicular names (Abhishek *et al*., 2010). In Siddha medicine *A. paniculata* used widely to treat fever like ckikunguny, swine –flu, typhoid, snake bite and common cold etc. (Dhiman *et al.*, 2012). It is an annual herb. The leaves are used traditionally in Asian traditional medicine and particularly in Ayurveda for treatment of various diseases and illness. The plant is cultivated in many areas, as well. It grows well in a sunny location. The seeds are sown during May and June.

A. paniculata grows erect to a height of 30 - 110 cm in moist, shady places. The slender stem is dark green. The lance-shaped leaves have hairless blades measuring 8 cm long by 2.5 wide. The small flowers are borne in spreading racemes. The fruit is a capsule around 2 cm long and a few millimeters wide. It contains many yellow-brown seeds.

A. paniculata is also used for other medical purpose; for example, digestive problems, blood cleanser, fever, sore throat (Sharma and Joshi, 2011). *A. paniculata* is used to cure fever and cold (Koul and Kapil, 1994). It is one of the best anti-malarial agent compared to the commercial product of quinine (Parvataneni et al., 2010). The herb has shown an ability to reduce inflammation (heat) and fight viral infections and is used as a principal ingredient in traditional Chinese medicinal formulas for lung support from colds (Sheeja *et al*., 2006). *A. paniculata* is a blood purifier, so it is used to cure turbid liver, jaundice, dermatological diseases, dyspepsia, febrifuge and anhelhemic. *A. paniculata* acts to dispel heat and remove toxin. Andrographaloid was found to be more potent and a standard hepato protective agent (Visen *et al.*, 1993). The whole plant of *A. paniculata* is used extensively as an anti-inflammatory and antipyretic drug for the treatment of laryngitis, diarrhea. The juice of fresh leaves generally contains andrographolide. It is used as a domestic remedy in the treatment of colic pain, loss of appetite, irregular stools and diarrhea (Mishra *et al.*, 2007). Since ancient times, *A. paniculata* has been known in traditional Asian medicine as an immune system booster, to treat infections in the gastrointestinal tract and upper respiratory tract, harps, sore throat and a variety of other chronic infectious diseases (Wangboonskul *et al.*, 2006). Nowadays microbes are resistance to various antibiotics. The resistant of microbes is due to indiscriminate utilization of commercial antimicrobial medicines supported by many scientists investigation for modern antimicrobial substances from several medicinal plants (Alagesaboopathi and Kalaiselvi, 2012). Most of the researchers concentrate on screening and minimum inhibitory concentration (MIC) of extracts of plants rather than identifying compounds with activity. In this attempt, we isolated active constituents of the plant and screened for antimicrobial activity which can be used further in research

to develop antimicrobial compounds with the isolated compounds or their synthetic analogues reported by Monoharan & Monoharan (2013). The present investigation was carried out to study the antibacterial potential of extract of *A. paniculata* against selective human pathogens.

II. MATERIAL AND METHODS

A. *Collection of plant material*

The whole plant of *A. paniculata* was collected from the College campus and verified by Dr. A.A. Khan, Retd. Prof. of Botany. Fresh and healthy leaf, stem, and root were used to extract bioactive fractions of *A. paniculata*. The parts of plants which were used for the extract were washed with water to remove soil and dust particles. Then they were dried under shaded place. Dried materials were blended to form a fine powder and store in airtight bottles (Sharma and Joshi, 2011).

B. *Test organisms*

The human pathogens *viz*., *Staphylococcus* sp., *E. coli, S. Typhi* and *Pseudomonas* sp., were collected from Govt. Kushabhau Thakare District Hospital Shahdol (M.P.) India. The test culture was maintained in Nutrient agar slant at 4°C for further studies.

C. *Preparation of plant extract*

The shade dried coarse powder of the leaf stem and root of *A. paniculata* were extracted using 250 ml solvent of ethanol, acetone, methanol and water with the help of soxhlet apparatus. The extracts were auto-claved to determine the stability of the crude extracts at the temperature of 121°C for 15 min and then the extracts were stored at 4°C for further use (Daniyan and Mohammed, 2008).

D. *Assay of antibacterial activity using the agar well diffusion method*

An agar-well diffusion method was employed for determination of antibacterial activities. The freeze- dried extract samples of spices and herbs were dissolved in phosphate buffered saline (PSB, pH 7.0 to 7.2) to the final concentration of 100 mg/ml and sterilized by filtration through 0.22 µm sterilized Millipore express filter. All bacteria were suspended in sterile water and diluted to 10-6 CFU/ml. The suspension (100 µl) was spread onto the surface of NA medium. Agar wells (4.6 mm in diameter) were cut from the agar with a sterile borer and 60 µl extract solutions were delivered into them. The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone of the tested bacteria.

III. RESULTS AND DISCUSSION

The antibacterial activity of different plant parts of *A. paniculata viz.*, root, stem and leaf were investigated using agar well diffusion method against some of the selected human pathogens such as *Staphylococcus* sp., *E. coli, S. Typhi*, and *Pseudomonas* sp. The extracts were prepared using various solvents such as ethanol, acetone, methanol and water. All the examined extract showed antibacterial activity against human pathogens (Table 1). Figure 1 depicts the antibacterial activity of leaf extract of *Andrographis paniculata* against *E. coli.*

Table 1. Antibacterial activity of Andrographis paniculata active against selective human pathogens.

S.No.	Plant parts of Andrographis paniculata	Solvent used	Zone of inhibition (mm.) of human pathogens			
			Staphylococcus sp.	E. coli	Slmonella typhi	Pseudomonas sp.
1.	Root	Ethanol	11.3	16.7	15.2	11.2
		Acetone	12.7	17.3	18.7	14.3
		Methanol	13.2	22.8	18.7	13.8
		Water	10.3	14.7	11.7	11.1
2.	Stem	Ethanol	8.7	11.2	23.3	9.1
		Acetone	13.4	17.6	19.4	9.2
		Methanol	16.6	29.2	18.8	19.6
		Water	10.2	10.3	13.3	7.5
3.	Leaf	Ethanol	9.2	19.4	25.2	18.9
		Acetone	14.9	25.2	28.6	21.5
		Methanol	18.4	32.8	24.7	24.2
		Water	8.9	11.9	11.9	16.1

The antibacterial activity of *A. paniculata* root extract of ethanol showed maximum zone of inhibition (16.7 mm) for *E. coli*, the minimum zone of inhibition (11.2 mm) for *Pseudomonas* sp. Then the activity of root extract of acetone showed maximum zone of inhibition (18.7 mm) for *Salmonella typhi*, the minimum zone of inhibition (12.7 mm) for *Staphylococcus* sp. The activity of root extract of methanol showed maximum zone of inhibition (22.8 mm) for *E. coli* and the minimum zone of inhibition (13.2 mm) for *Staphylococcus* sp. Then, the solvent of water showed maximum zone of inhibition (14.7mm) for *E. coli* and the minimum zone of inhibition (10.3 mm) for *Staphylococcus* sp.

The activity of stem extract of ethanol showed maximum zone of inhibition (23.3 mm) for *S. Typhi*, and minimum zone of inhibition (8.7 mm) for *Staphylococcus* sp. Then the activity of stem extract by acetone showed maximum zone of inhibition (19.4 mm) for *S. Typhi* and the minimum zone of inhibition (9.2 mm) for *Pseudomonas* sp. The activity of stem extract of methanol showed maximum zone of inhibition (29.2 mm) for *E. coli* and the minimum zone of inhibition (16.6 mm) for *Staphylococcus* sp., the antibacterial activity of stem extract of water showed maximum zone of inhibition (13.3 mm) for *S. Typhi*, and the minimum zone of inhibition (7.5 mm) for *Pseudomonas* sp.

The activity of leaf extracts of ethanol showed maximum zone of inhibition (25.2 mm) for *Salmonella typhi* and the minimum zone of inhibition (9.2 mm) for *Staphylococcus* sp. Then the activity of leaf extracts of acetone showed maximum zone of inhibition (28.6 mm) for *S. Typhi* and the minimum zone of inhibition (14.9 mm) for *Staphylococcus* sp. The activity of leaf extracts of methanol showed maximum zone of inhibition (32.8 mm) for *E. coli* and the minimum zone of inhibition (18.4 mm) for *Staphylococcus* sp. The antibacterial activity of leaf extracts of water showed maximum zone of inhibition (11.9 mm) for *Salmonella typhi* and *E. coli* the minimum zone of inhibition (8.9 mm) for *Staphylococcus* sp.

The result reveals that methanol based leaf extract was more effective when compared with other extracts and for all solvent used, methanol was best. So, further, minimum inhibitory concentration was studied for methanol extract of leaves. The different concentrations *viz*., 0, 25, 50, 75 and 100 µl were taken. Then, the well diffusion method was followed for all the pathogens (Table 2).The result reveals that 75 µl was optimum for all the test cultures and it was found to have more activity for *E. coli* (32.3 mm) followed by *S. Typhi*, (28.1 mm), *Staphylococcus* sp., (13.1 mm) and *Pseudomonas* sp., (14.1 mm). Figure 2 depicts the minimum inhibitory concentration of methanolic leaf extract of *A. paniculata* against *E. coli.*

IV. DISCUSSION

Infectious diseases are a major cause of morbidity and mortality worldwide. Currently, the ongoing battle against bacteria prevails certainty of evolving, resistance. On the other hand, advancement in medical field results in more patients being in critical and immune suppressed states, thus creating a perpetual need for new antibiotics. As a result, it is the right time to discover new antibiotics (Mahesh and Satish, 2008). *A. paniculata* has a several water soluble lactone andrographoloidic properties. Medicinal plants are more important in field of pharmaceutical industries for new drug preparation (Sule *et al.*, 2010).

Table 2. Minimal Inhibitory Concentration of *Andrographis paniculata.*

Methanol (µl)	Zone of inhibition of the human pathogens			
	Staphylococcus sp.	*E. coli*	*Slmonella typhi*	*Pseudomonas sp.*
0	10.0	25.3	23.7	11.3
25	11.1	26.0	25.3	12.3
50	12.0	26.8	25.6	13.0
75	14.1	32.3	28.1	13.4
100	13.3	32.5	28.8	14.5

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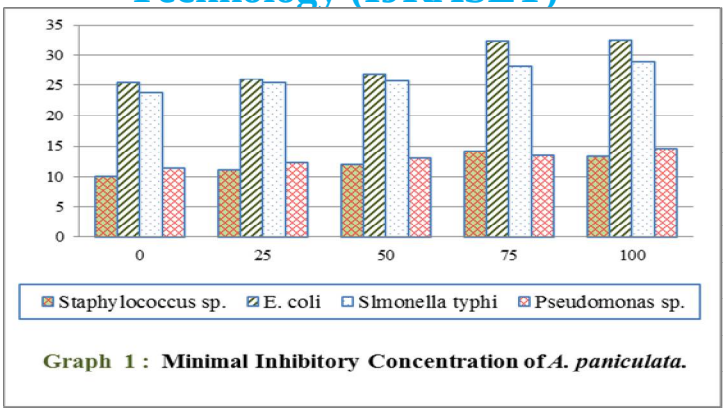

Graph 1 : Minimal Inhibitory Concentration of *A. paniculata.*

Maximum zone of inhibition was recorded with 75 µl methanol extract against *S. aureus*, in accordance with the previous studies reporting that 75 µl methanol is better than other solvent for antibacterial activity (Pushpendra *et al.*, 2013). Therefore, only the 75 µl of methanol extract of *A. paniculata* leaves were used for further experiments. The present study mainly focused on assaying the efficiency of different plant parts *viz*., root, stem and leaf of *Andrograpish paniculata* using various solvent extraction procedures against the selected human pathogens.

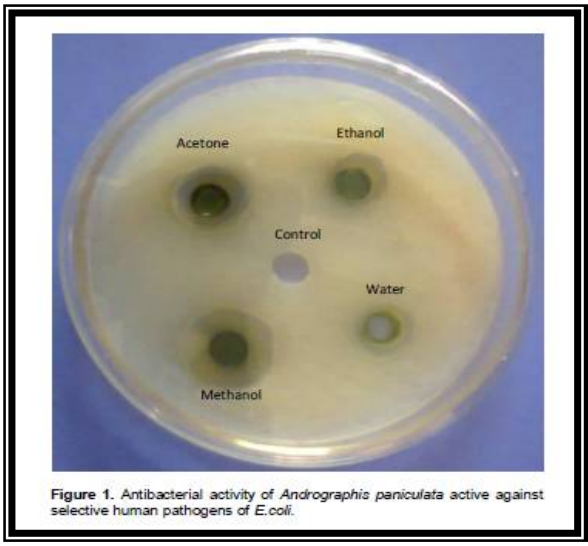

Figure 1. Antibacterial activity of *Andrographis paniculata* active against selective human pathogens of *E.coli*.

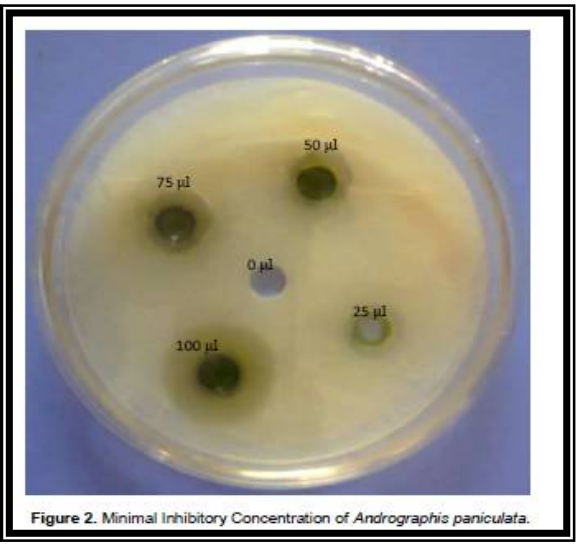

Figure 2. Minimal Inhibitory Concentration of *Andrographis paniculata*.

In the preliminary screening of antibacterial activity of methanol leaf extract of *Andrographis paniculata* exhibited maximum activity when compared with other plant parts and also from different solvent extracts (Monoharan and Monoharan, 2013). The maximum activity was observed for the pathogens *E. coli* followed by *Salmonella typhi, Staphylococcus* sp. and *Pseudomonas* sp.

The methanolic extracts of *Andrographis paniculata* at the highest concentration showed the strongest bacterial inhibitory activity of other extracts. This similar observation reported by many researchers (Negi *et al.*, 2005; Parekh and Chanda, 2010; Al-Bayati, 2008; Kaushik and Goyal, 2011).

The methanol leaf extract was studied for minimal inhibitory concentration concept for various concentra-tions *viz*., 0, 25, 50, 75 and 100 µl. Among the different concentrations, 75 µl showed maximum activity for all the pathogenic organisms and recorded highest for *E. coli* (32.3 mm) and it was on par with 100 µl concentrations. From the result, it was revealed that plant extracts of *A. paniculata* showed strongest antimicrobial activity for a wide range of pathogens and also more reliable than commercially available antibiotics.

V. CONCLUSION

A. paniculata has been used in Ayurveda, Unani and Siddha systems of medicine from ancient times. It has wide spectrum of pharmacological activities either in the form of powder, extracts or in its isolated compounds with minimum side effects; several products fortified with extract or isolated compounds have been launched in national and international markets for various diseases. In this context, the present study was carried out to find out the antibacterial potential of various solvent based extract of *A.*

paniculata against selective human patho-gens. The antibacterial activity of *A. paniculata* may be due to the presence of active principle called andrographoloid. In future, the improvements of active principle andrographoloid content in *A. paniculata* using plant growth promoting rhizobacteria (PGPR) are studied. The enriched *A. paniculata* plant extracts are further studied for their antibacterial properties against selective human pathogens.

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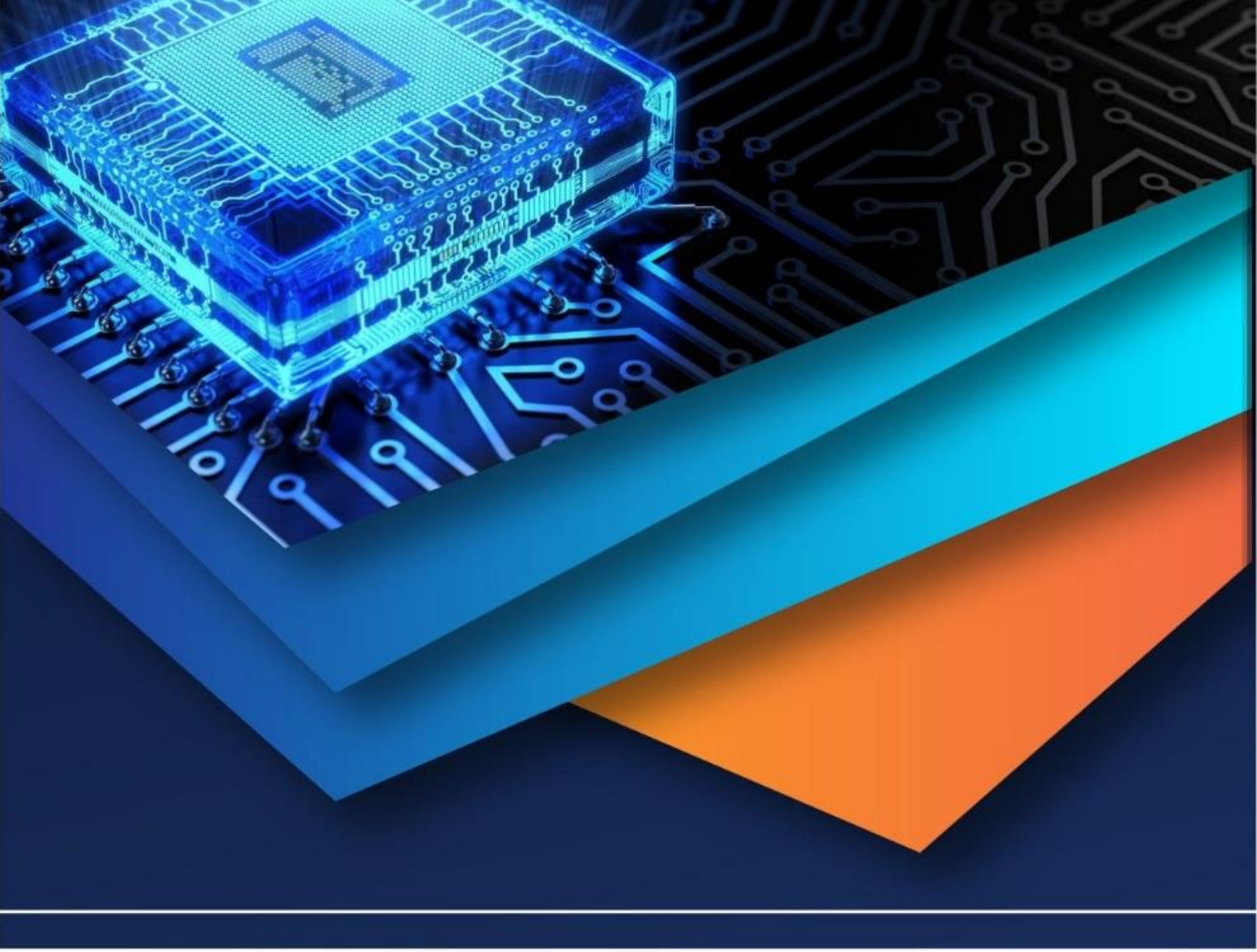

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