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Study Of Antimicrobial Activity Of *Andrographis Paniculata* Activity, Esbl, Resistotyping, Uropathogens

KEYWORDS: Andrographis paniculata, Antimicrobial activity, ESBL, Resistotyping, Uropathogens

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ABSTRACT *Pathogenic microorganisms show higher degree of resistance to antibiotics. The resistance to the beta lactam antibiotics shows the complications as MRSA, VRE and ESBL. To screen bioactive phytochemicals for their antimicrobial activity can be the remedy for this. Andrographis paniculata extracts (aqueous and organic solvent extracts viz. ethanol, chloroform, IPA, acetone, Ethyl acetate) were tested against three multi- drug resistant uropathogenic E. coli, UPEC2 (100% resistance), UPEC3 (94.11% resistance), and UPEC4 (88.23% resistance). After testing these uropathogens with Andrographis paniculata, maximum antimicrobial activity (75%) was given by the aqueous extract which show zone of inhibition for three E. coli cultures and the type strain also. The ethanol and ethyl acetate extract shows 50% occurrence for zone of inhibition. The acetone and IPA extract shows 25% positive results whereas the chloroform and petroleum extract was unable to express any antimicrobial activity.*

INTRODUCTION:

Various pathogenic microorganisms show higher degree of resistance to beta lactam antibiotics e.g. Penicillins, cephalosporins, cephamycins etc by producing Beta Lactamases enzyme that inactivates the structure of these antibiotics (Lakshmi et. al. 2014). The resistance to the beta lactam antibiotics shows the complications as MRSA (Mithicillin Resistance S. aurous, VRE (Vancomycin Resistant Enterococci) and ESBL (Extended Spectrum Beta Lactamases). Amongst the above complications ESBL is the most sensational issue now a day due to the occurrence of Superbug, NMD1(New Delhi Metallo betalactamases 1) which shows resistance to imipenem, which is a mainstay for the treatment of antibiotic-resistant bacteria. (Kumarasamy *et al* 2010). Bacteria which carry such genes are often referred to in the news media as "Superbugs", since infections with these bacteria are very hard to treat successfully using the available antimicrobial therapy because of its drug resistance problem and side effects. Hence to derive or screen for the new molecules having the same therapeutic activity with lower adverse effects, higher efficacy and potency is the need of time. One of the promising approaches to tackle the problem is to screen bioactive phytochemicals for their antimicrobial activity.

Andrographis paniculata Nees is an herbaceous plant in the family Acanthaceae, native to India and Shrilanka and commonly known as kalmegh. It is widely cultivated in southern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes.

Andrographis paniculata is an erect annual herb extremely bitter in taste in all parts of the plant body. The plant is known in north-eastern India as Maha-tita, literally "king of bitters". Andrographis paniculata grows erect to a height of 30–110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 centimeters long by 2.5 wide. The small flowers are borne in spreading racemes. The fruit is a capsule around 2 centimeters long with many yellow-brown seeds. The herb is the well-known drug Kalmegh 'green chiretta', and forms the principal ingredient of a household medicine ('alui'), used as a bitter tonic and *febrifuge*. The Tamils have been using Nilavempu - as it is called in Tamil - for centuries. In Siddha medicine, Andrographis Paniculata is used widely to treat fevers like chikenguinea, swine-flu, typhoid etc.

Andrographis paniculata plant extract is known to possess a variety of pharmacological activities. The therapeutic value of Kalmegh is due to its mechanism of action which is perhaps by *enzyme induction* (Niranjan *et al* 2010). It is important in the treatment of gastrointestinal, upper respiratory infections, fever, harpies, sour throat, hepatitis, and variety of other chronic infections. The plant shows antibacterial, anti malarial, anti diarrheal, filairicidal, cardiovascular, fertility effects and protection of liver and gall bladder (Chopra *et al* 1956). The herb and its isolates like andrographolide *Andrographolide* are reported to pusses anti inflammatory, hepatoprotective, astringent, anodyne, tonic, alexipharmic and antipyretic activities. (Prajapati N. D. *et al* 2003).

The specified plant leaves are here being used as putative plant part in this study. The aim of this study was to appraise the possible antibacterial potential of *Andrographis paniculata* extracts (aqueous and organic solvent extracts viz. ethanol, chloroform, IPA, acetone, Ethyl acetate) against multi- drug resistant bacterial pathogens. For this purpose both clinical isolates i.e. ESBL producing uropathogenic Escherichia coli and standard type control strain was tested in this study. Antibacterial potency of the extracts was tested by agar diffusion assay as growth inhibitory assay methods.

MATERIALS AND METHODS:

1. Photochemical study:

Plant material:

The leaves were studied as the medicinal plant parts and

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were collected from the local market in Aurangabad in March, 2011.

Preparation of herbal specimens:
All the plant material was shade dried for 10 days. Five hundred grams of dried plant material was crushed directly by grinder without adding any solvent.

a. Aqueous extracts
Fifty grams of above mentioned dried, powered herbal material was weighed and added to 50 ml of cold distilled water into a conical flask stoppered with rubber corks and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask and subjected to drying. The extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test.

b. Organic solvent extracts:
To prepare organic solvent extract, 25 g powder of Andrographis paniculata leaves was kept in each solvent for consecutive 3 days at room temperature and filtered. The filtrate was centrifuged at 3000 rpm for 15 min and evaporated to dryness in a water bath. All the aqueous and organic extracts were stored at 4°C in air-tight jars until further use. (Chattopadhyay *et al*. 2009).

c. Formulation of extracts:
Organic Solvent extract was reconstituted in 5% Di methyl sulfoxide (DMSO) in water and aqueous extract in sterile distilled water to a final concentration of 100 mg/ml.

2. Microbiological study:
a. Test organisms:
The E.coli culture ATCC 25992 was used as reference strain. The type strain was procured from National Collection of Industrially important microorganisms NCIM, NCL, Pune India. The clinical urinary pathogens were procured from M. D. Services Pvt. Ltd., Aurangabad.

b. Resistotyping and ESBL detection:
The clinical isolates were resistotyped using CLSI recommended antibiotics (Table 1). These isolates were then also confirmed for their ESBL production using ESBL determination Kit (Himedia Ltd. Mumbai, India.) containing Ceftazidime and ceftazidime Clavulunic acid. All the tested strains were maintained in nutrient agar slants at 4°C.

c. Identification of clinical isolates:
The clinical isolates were identified using conventional biochemical tests and differential agar like Mac'conkey agar, TSI, EMB, Phenyl alanine agar etc.

3. Antimicrobial Activity study:
a. Inoculum preparation:
Susceptibility tests were performed by a modified agar well diffusion method (Chattopadhyay R. R. *et al*. 2009). The inoculum size of the test strains were standardized according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 1993). The bacterial strains were inoculated in Mueller Hinton Broth (Hi-media, Mumbai, India) and incubated at 37°C in a shaker water bath for 3 - 6 hrs.

b. Determination of inhibitory zone diameter (IZD):
One ml of standard suspension of each bacterial strain was spread evenly on Mueller-Hinton Agar (Hi-Media, Mumbai, India) plates using a sterile glass rod spreader and the plates were allowed to dry at room temperature. Subsequently six mm diameter wells were bored in the agar and 100 µl volumes of 100 mg/ml of each reconstituted extract was pipetted into wells. After allowing the diffusion of extract into the agar, for 2 hrs, the plates were incubated at 37°C for 24 hrs. Inhibition Zone Diameter (IZD) was measured to the nearest millimetre. (Table II). Ceftazidime-Clavulunic acid disc (Hi-media, Mumbai, India) (10 µg/ml) was used as experimental positive control and 5% DMSO as negative control. The tests were performed in triplicate for each microorganism used and the final results were expressed as the arithmetic average of triplicate experiments.

Results:
All the clinical isolates procured were resistotyped (Table no. 1 about here) and found to multiple antibiotic resistant. Amongst them isolate UPEC2 shows 100% resistance, UPEC3 shows 94.11% resistance, and UPEC4 shows 88.23% resistance to the antibiotics used for screening. The maximum resistance (100%) was exhibited to ampicillin, Mithicillin, pipracillin, carbancillin, nalidixic acid, ciprofloxacin, ofloxacin, sparfloxacin, cefuroxime, ceftazidime, gentamycin, chloramphenicol, cortimoxazole and vancomycin where as amoxicillin, lomfloxacin and nitrofurantoin sensitivity was shown by one culture each. These pathogens were checked for their ESBL Nature (Table no. 2 about here) and found to be ESBL positive. They are identified biochemically as Escherichia coli.

Various extracts were obtained and were cconsidered for their weigh and color characterization (Table no. 3 about here).The selected cultures were resistotyped using various extracts of Kalmegh. (Table no. 4 about here). These clinical isolates were further analysed using various extracts of Andrographis paniculata. All the tested extracts showed to varying degrees of strain specific antibacterial potential against tested strains. Maximum activity (75%) was given by the aqueous extract which show zone of inhibition for three E. coli cultures and the type strain. The ethanol and ethyl acetate extract shows 50% occurrence for zone of inhibition. The acetone and iso propyl alcohol extract shows 25% positive results whereas the chloroform and petroleum extract was unable to express any antimicrobial activity.

Conclusions:
Infectious diseases are the world's leading cause of premature deaths. In recent years, drug resistance to human pathogenic bacteria has commonly been reported from all over the world (Piddock, 1989). Uropathogens have developed high level of resistance to first line agents used against treatment. Even though pharmaceutical companies produced a number of new antibacterial drugs, resistance to these drugs by bacteria increased and became a global concern. MRSA and multi-drug resistant as well as ESBLs producing uropathogenic E. coli have been recognized as the major causes of infections in humans (Solby 2005). Therefore, the importance of identifying new effective antimicrobial agents cannot be overemphasized.

The increasing interest on traditional ethno-medicine may lead to discovery of novel therapeutic agents. Also India is a great repository of medicinal prosperity. Medicinal plants inhabitant to this region. They are finding their way into pharmaceuticals, cosmetics and food supplements. The World Health Organization has estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs. It is getting popularized in developing and developed countries owing to its natural origin and lesser

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side effects. The use of plant extracts or phytochemicals with known antimicrobial properties can be of great consequence of therapeutic treatments. The plant Andrographis paniculata was investigated for many of its therapeutic properties viz. antiinflammatory, antipyretic, antibacterial, antimalarial, antidiarrhoeal, filairicidal, cardiovascular, fertility effects and been reviewed by Niranjan A *et al* in 2010. Singha *et al* in 2003 have reported the antimicrobial activity of aqueous extract of Kalmegh. The antimicrobial activity of the ethanoic extract was found to inhibit the adherence of Streptococcus mutants was studied by Limsong *et al* in 2004. Sharma A. *et al* in 2009 have found the maximum antimicrobial activity in ethanol and acetone extract of whole processed plant against urinary tract pathogens as E.coli, proteus sp., Klebsiella sp. etc.

The Preliminary results of this investigation indicate that leaves have the potential of microbial inhibition. The results of present study conclude that the plant Andrographis paniculata and its aqueous extracts mainly give the antimicrobial activity against MDR, ESBL producing E. coli.

Further work would be done to locate the active principle from the various extracts and their phytopharmaceutical studies. The study of local medicinal plants is expected to increase the use of these plants in the therapy against infections caused by the MDR, ESBL bacteria. It is possible that enhanced therapy for many diseases can be found during this study.

Table 1: Resistotyping Analysis:

Sr. No.	Culture no.	A	Am	M	Pc	Cb	Nx	Cf	Of	Lo	Sp	Cu	Cft	G	C	Va	Co	Nf	Total R	%
1	UPPR 1	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	14	82.35
2	UPEC 2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	17	100
3	UPEC 3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	16	94.11
4	UPEC 4	R	S	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	15	88.23
5	UPPS 6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	17	100

Table 2: Table: Identification and ESBL confirmation of Clinical isolates:

Sr. No.	Culture no.	Identification	ESBL nature
01	UPEC 2	E. coli	Positive
02	UPEC 3	E. coli	Positive
03	UPEC 4	E. coli	Positive

Table 3: Phenotypic appearance of extracts of Andrographis paniculata leaves powder:

Plant name	H_2O		ET		AC		IPA		PE		EA		$CHCl_3$	
	% Yield	color	% Yield	color	% Yield	color	% Yield	color	% Yield	Color	% Yield	color	% Yield	color
Kalmegh	2.345	Colorless	3.230	Reddish	07.98	Brown	08.73	Black	08.67	Reddish	00.23	Brown	07.05	Yellow

Table 4: Antibacterial potential of extracts of Andrographis paniculata (leaves):

Sr. No.	Culture	Andrographis paniculata leaves								DMSO	CEC
		ETH	ACE	IPA	EA	H_2O	$CHCl_3$	PE	% sensitivity		
1	UPEC 2	R	S	R	R	R	R	R	14 %	--	19
2	UPEC 3	R	S	R	S	S	R	R	43 %	--	25
3	UPEC 4	S	R	S	R	S	R	R	29 %	--	23
4	ATCC 25992	S	S	R	S	S	R	R	57 %	--	26
		50%	25%	25%	50%	75%	00%	00%			

ETH: Ethanol Extract ACE: Acetone Extract IPA: Iso-propanol Extract EA: Ethyl Acetate Extract

H_2O: Cold water Extract HCl_3: Chloroform Extract PE: Petroleum Ether Extract Extract conc.: 100mg/ml in DMSO
+: zone of inhibition --: no zone of inhibition: UPEC: Uropathogenic Escheriatia sp. ATCC 25992: Type sensitive E. coli Strain

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