Research Paper

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Antimicrobial activity of Tribulus terrestris against urinary pathogens exhibiting ESBLs.

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KEYWORDS : Antimicrobial activity, ESBL, Resistotyping, Tribulus terrestris, Uropathogens.

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ABSTRACT

The increasing degree of antibiotic resistance exhibited by pathogenic microorganisms is a cause of concern. Since being a drug of choice the resistance shown for beta lactam antibiotics is of greater significance. Amongst the ways available to overrule this problem, usage of bioactive phytochemicals is a significant solution. The plant Tribulus terrestris, which is widely available in most of the regions of world, is thus screened in this study. Tribulus terrestris 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 Tribulus terrestris, Maximum activity (75%) was given by the ethanol extract which show zone of inhibition for three E. coli cultures and the type strain. The acetone extract shows 50% occurrence for zone of inhibition. The acetone, ethyl acetate, iso propyl alcohol and petroleum ether extract shows 25% positive results whereas the chloroform extract was unable to express any antimicrobial activity.

INTRODUCTION:

In the past, plants provided a source of inspiration for novel drug compounds, as plant-derived medicines made large contributions to human health and well being. Globalization interferes with infectious disease control at the national level while microbes move freely around the world. After the occurrence of ESBL Superbug, NMD1 (New Delhi Metalobetalactamases1) ESBL treatment is found to be more critical (Kumarasamy *et al.* 2010). Hence to screen for the new molecules having the same therapeutic activity with higher specificity, lower adverse property and higher potency becomes necessary.

The study is thus based on screening of various extracts of *Tribulus terrestris* against ESBL pathogens. *Tribulus terrestris Linn.* (Gokhru and Bhakra) is an annual or biennial, prostrate herb, distributed in tropical and subtropical countries in Asia, Africa, S. Europe and North Australia. Flowers are perfect and regular; sepals imbricate or valvate, free, persistent or deciduous; petals usually free and imbricate. Disk or nectary glands are either present or absent. The ovary is superior, 2 to 5 or 10-lobed, and fruit capsule is often spiny or tuberculate (Dastagir 2012).The fruits are used in urinary bladder, while leaves are used in colic and chronic cough (Marwat *et al.*, 2008). Presently, preparations and the dietary supplements containing extracts of *T. terrestris* are used for sexual impotence, edema, skin diseases, vermifuge, rheumatoid arthritis and others (Semerdjieva, 2011). The aim of this study was to appraise the possible antibacterial potential of *Tribulus terrestris* extracts (aqueous and organic solvent extracts viz. ethanol, chloroform, IPA, acetone, Ethyl acetate) against multi- drug resistant bacterial pathogens. Antibacterial potency of the extracts was tested by agar diffusion assay as growth inhibitory assay methods using test and standard strains.

MATERIALS AND METHODS:

Photochemical study:

Plant material:

The fruits were studied as the medicinal plant parts and were collected from the local market in Aurangabad in March, 2011. All the plant material was shade dried for 10 days.

Aqueous extracts

25gms 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 3 days with occasional shaking. Filtered off using sterile Whattman filter paper and subjected to drying. The extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test.

Organic solvent extracts:

To prepare organic solvent extract, 25grams powder of *Tribulus terrestris* fruits 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 airtight jars until further use. (Chattopadhyay *et al.* 2009).

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.

Microbiological study:

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.

Resistotyping and ESBL detection:

The clinical isolates were resistotyped using CLSI recommended antibiotics (Table 1). These isolates were confirmed for their ESBL production using ESBL determination Kit (Himedia Ltd. India). All the tested strains were maintained in nutrient agar slants at 4°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.

Antimicrobial Activity study:

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 NCCLS guidelines (NCCLS,1993). The bacterial strains were inoculated in Mueller Hinton Broth (Hi-media, India) and incubated at 37°C in a shaker water bath for 3 - 6 hrs.

Determination of inhibitory zone diameter (IZD):

Suspension of each bacterial strain was spread evenly on Mueller-Hinton Agar (Hi-Media, India) plates. 6 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. Ceftazidime-Clavulunic acid disc (Hi-media, India) was used as experimental positive control and 5% DMSO as negative control.

Results:

All the clinical isolates procured were resistotyped (Table no. 1 about here) and found as 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 and found to be ESBL positive. They are identified biochemically as *Escherichia coli* (Table no. 1 about here).

Various extracts were obtained and were cconsidered for their weight and color characterization (Table no. 2 about here).The selected cultures were resistotyped using various extracts of *Tribulus terrestris*. (Table no. 3 about here). These clinical isolates were further analysed using various extracts of *Tribulus terrestris* **.** All the tested extracts showed to varying degrees of strain specific antibacterial potential against tested strains. Maximum activity (75%) was given by the ethanol extract which show zone of inhibition for three *E. coli* cultures and the type strain. The acetone extract shows 50% occurrence for zone of inhibition. The acetone, ethyl acetate, iso propyl alcohol and petroleum ether extract shows 25% positive results whereas the chloroform extract was unable to express any antimicrobial activity.

Conclusions:

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 available antibiotics used against them. The use of plant extracts or phytochemicals with known antimicrobial properties can be of great consequence of therapeutic treatments. The plant *Tribulus terrestris* was investigated for many of its therapeutic properties viz. colic , chronic cough (Marwat et al. 2008), edema, skin diseases, vermifuge, rheumatoid arthritis (Semerdjieva, 2011). Raja M. and R. Venkatraman in 2011 have investigated the its antibacterial properties and found that aqueous extract is lacking any antibacterial potential as we have also found but unlike their results we have reported no antibacterial activity against the test bacteria in chloroform extract which they have found. Dastagir *et al* in 2012 have found the potent antibacterial activity of methanol against *Klebsiella pneumoni* and *S. aureus* whereas the n- hexane extract has shown the maximum activity against *E. coli*. The Preliminary results of this investigation indicate that leaves have the potential of microbial inhibition. Hence the present study concludes with the considerable antimicrobial potential of plant *Tribulus terrestris* against ESBL producing *E. coli*. Further work would be done to locate the active principle from the various extracts and their phytopharmaceutical studies.

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Table 1: Resistotyping Analysis:

Culture no.	A	Am	M	Pc	Cb	Nx	Cf	Of	Lo	Sp	Cu	Cft	G	C	Va	Co	Nf	Total R	%	E S B L nature	Identific-ation
UPEC 2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	17	100	+	E. coli
UPEC 3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	16	94.11	+	E. coli
UPEC 4	R	S	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	15	88.23	+	E. coli

Table 2: Phenotypic appearance of extracts of *Tribulus terrestris* 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
Gokhru	2.498	Brown	4.847	Brown	01.76	Greenish Brown	02.75	Reddish	05.89	Greenish Brown	02.97	Colourless	57.69	Colourless

Table 3: Antibacterial potential of extracts of *Tribulus terrestris:*

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

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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