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In Vitro Anti-microbial effect of various extracts of *Ġokṣura* (*Tribulus terrestris*) fruits on common pathogens causing Urinary Tract Infection

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ABSTRACT

Introduction: The present study was carried out with an objective to investigate the antimicrobial potentials of various extracts of *Gokṣura* (*Tribulus terrestris* Linn.) fruits on common uropathogen strains. **Material and Methods:** Aqueous, ethanol, chloroform, petroleum ether extracts of fruits of *Tribulus terrestris* were evaluated for potential antimicrobial activity against certain uropathogen strains. The antimicrobial activity was determined in the extracts using agar well diffusion method. The antibacterial activities of extracts (5%, 10% and 15% w/v) of *Tribulus terrestris* were tested against *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Enterococcus faecalis.* Zone of inhibition of extracts were compared with that of standard drug Azithromycin 1 % w/v (Positive control) and DMSO(Negative control) for antibacterial activity. **Observations and Results:** Inhibition of the bacterial growth was shown against the tested organisms in all extracts but ethanol extract at 15% concentration showed highest activity against all pathogens. The phytochemical analyses of the plants were also carried out which was found to be similar to standard values of API. **Conclusion:** The results of this study showed that *Tribulus terrestris* possesses significant antibacterial activity against common uropathogens.

Key words: *Tribulus Terrestris, In Vitro Antimicrobial Activity, UTI, Uropathogens, In Vitro Study.*

INTRODUCTION

Urinary tract infection (UTI) is the second most common bacterial infections worldwide after respiratory infections. It is defined as microbial infiltration of urinary tract and it encompasses infections of the urethra (urethritis), bladder (cystitis), ureters (ureteritis), and kidney (pyelonephritis).[1] The effect of UTI ranges from a mild self-limiting sickness to acute sepsis, with a mortality rate of 20-40%,[2] which increases inexplicably with age. As UTI is generally caused by bacteria, they are most frequently treated with antibiotics. The type of medication and length of treatment depends on type of bacteria, its level of susceptibility, history, symptoms, and immune status of the patient. To treat chronic and recurrent UTI, different methods are practiced like antibiotics, bioactive natural foods, using probiotics, and maintaining good personal hygiene, but still, the problem is yet to be addressed successfully.

As stated above antibiotics are frequently used to treat and prevent acute and recurrent UTI, but their repeated use often results in dysbiosis of vaginal and intestinal normal flora, as well as antibiotic resistance due to the high mutation ability and horizontal gene transfer capability of different pathogens. UTIs are becoming increasingly difficult to treat owing to the rapid spread of drug resistance among Gram-negative

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organisms, including UPEC.[3] Different mechanisms are used by uropathogens for survival in the bladder under stresses such as starvation and immune responses.[4] Uropathogens undergo morphological changes, invade uroepithelial cells, and form biofilms to persist and cause recurrent infections. Extracellular DNA, exopolysaccharides, pili, flagella, and other adhesive fibers create a niche for a bacterial community that is secluded from antimicrobial agents, immune responses, and other stresses. These factors results in emergence of antibiotic-resistant bacterial pathogens and leads to the spread of antibiotic resistance. So, there is need of alternative methods for the prevention and treatment of UTIs.[5] Several in vitro and in vivo studies have reported urobactericidal activity of certain herbal drugs. In the current research study, screening of fruits of *Tribulus terrestris* was done in order to explore new source of antimicrobial agent.

Materials and Methods

Test Sample

Fruits of *Gokṣura* (*Tribulus terrestris)* was purchased from local market and the sample was identified and authenticated by CSIR- National Institute of Science Communication and Information Resources, Raw material Herbarium and Museum, New Delhi (vide reference number NISCAIR/RHMD/Consult/2020/3708-09-1 to 5 on date 18/12/2018).

Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literatures.

Preparation of different extracts

The extraction of the *Tribulus terrestris* fruits was carried out by using known standard procedures. Aqueous, Ethanol, Chloroform, Petroleum ether extracts were prepared by soxhlet extraction method and removal of solvent was done in rotary evaporator. 5%, 10% and 15 % w/v solution were prepared using Dimethyl sulfoxide (DMSO).

Test Microorganisms and Growth Media

Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 12453), *Klebsiella pneumoniae.* (MTCC 4030), *Enterococcus faecalis* (MTCC 439) were chosen based on their clinical importance in UTI. The bacterial cultures were incubated for 24 hours at 37°C on nutrient agar and were then stored at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C. The stock cultures were maintained at 4°C.

Antimicrobial Activity

Agar well diffusion method was chosen for in vitro anti microbial study as it is precise and reliable method. Autoclaved agar media (20 ml) was poured into each petri plate, followed by the swabbing of bacterial colony from the inoculums of the test microorganisms on prepared media plates with the use of a sterile stainless steel borer, wells (with a diameter of about 5mm) were drilled into the plates. Using sterile syringes, plant solvent extracts were injected into the designated wells. The plates were then kept in an incubator at 37°C for 24 hours. Subsequently dimethyl sulphoxide-DMSO 0.1%- (the solvent used to reconstitute the test sample) was also poured to assess its activity, if any (as Negative Control) and standard antibiotic disc- Azithromycin 1 % w/v (Positive control) was placed in the same plate. All the plates were incubated at 37°C for 24 h.

Determination of zone of inhibition method

Each plate was inspected after incubation for anti bacterial activity. The diameter of the well as well as the diameter of the zones of absolute inhibition were measured and recorded to the nearest whole millimetre. The experiment was done in triplicate, average diameter of the zone of inhibition was measured in millimeters by the help of the scale and then mean was calculated. The activity index was

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calculated from the mean of the three measurements by using formula as follows

Determination of the activity index[6]

The activity index of the test samples extract was calculated as

$$\text{Activity index (AI)} = \frac{\text{Zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

Observations and Results

Preliminary phytochemical screening

All the observed values of phytochemical study were corresponding with the standard values of API. Therefore, the drug samples of *Gokşura (Tribulus terrestris)* used in this study was of desired quality.

Anti Microbial Activity

For screening antibacterial activity using Agar well diffusion method against clinical isolates of five uropathogens.[7] Aqueous, Ethanol, Chloroform, Petroleum ether extracts were compared with the Positive Control (Standard drug Azithromycin 1 % w/v) and Negative control i.e., DMSO [Figures 1 to 5]. The results are presented in Table No. 1. & Table no. 2

Table 1: Mean of ZOI (in mm) of different extracts of *Gokşura (Tribulus terrestris)* against *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Enterococcus faecalis* with negative and positive control

Drug	Concentration	Zone of Inhibition (mm)				
		E. Coli	*P. aeruginosa*	*P. mirabilis*	*K. pneumoniae*	*E. faecalis*
Aqueous Extract of *Gokşura*	5% w/v	11	10	9	8	10
	10% w/v	13	13	12	10	13
	15% w/v	15	16	15	13	14
Ethanol Extract of *Gokşura*	5% w/v	14	13	12	14	13
	10% w/v	17	16	15	16	15
	15% w/v	20	18	19	18	19
Chloroform Extract of *Gokşura*	5% w/v	12	10	11	12	14
	10% w/v	14	14	14	16	16
	15% w/v	15	17	16	18	18
Petroleum ether Extract of *Gokşura*	5% w/v	9	11	8	10	13
	10% w/v	12	14	12	13	15
	15% w/v	16	15	14	15	17
Negative Control		00	00	00	00	00
Positive Control		31	28	25	27	29

Table 2: Activity index of different extracts of *Gokşura (Tribulus terrestris)* against trial pathogens in comparison to positive control

Pathogen	Concentration	Activity Index				
		E. Coli	*P. aeruginosa*	*P. mirabilis*	*K. pneumoniae*	*E. faecalis*
Aqueous Extract of *Gokşura*	5% w/v	0.35	0.36	0.36	0.30	0.34
	10% w/v	0.42	0.46	0.48	0.37	0.45
	15% w/v	0.48	0.57	0.60	0.48	0.48
Ethanol Extract of *Gokşura*	5% w/v	0.45	0.46	0.48	0.52	0.45
	10% w/v	0.55	0.57	0.60	0.59	0.52
	15% w/v	0.65	0.64	0.76	0.67	0.66

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Chloroform Extract of *Gokṣura*	5% w/v	0.39	0.36	0.44	0.44	0.48
	10% w/v	0.45	0.50	0.56	0.59	0.55
	15% w/v	0.48	0.61	0.64	0.67	0.62
Petroleum ether Extract of *Gokṣura*	5% w/v	0.29	0.39	0.32	0.37	0.45
	10% w/v	0.39	0.50	0.48	0.48	0.52
	15% w/v	0.52	0.53	0.56	0.55	0.59

All four extracts of *T. terrestris* (Aqueous, Ethanol, Chloroform, Petroleum Ether) at different concentrations (5, 10 and 15%) showed concentration dependent antimicrobial effect against all the five pathogens (*E. coli*, *P. aeruginosa*, *P. mirabilis, K. pneumonia, E. faecalis)*. It was observed that Ethanol extract at 15% concentration showed highest activity against all pathogens. All extracts at 15% concentration were found to be biologically active against all pathogens except aqueous extract which was found active against *P. aeruginosa, P. mirabilis* only.

Discussion

In the present study, we have used the Agar well diffusion method for the antibacterial evaluation of *T. terrestris*. There was a significant antibacterial activity of the test drug *T. terrestris* when it was compared with the standard drug Azithromycin 1 % w/v but the activity was different in four extracts. Activity was also different for the various micro-organisms evaluated. The difference in the activities may be due to type of solvent used in extract procedures. The variation in effectiveness of the extract against different microorganism may depend upon the chemical composition of the extract and membrane permeability of microbes for their chemical and metabolism.

The main components of *T. terrestris* are saponins and these compounds play critical role in antimicrobial activity of this plant. The antimicrobial activities of saponins were confirmed against different microorganisms in previous studies.[8,9] Saponins are detergent like substance and with surface active properties may disturb the bacterial membrane cells of bacteria.[10] *T. terrestris* contains lot of saponins and it also exhibited the high antibacterial activity against clinical isolates of microorganisms in this study.

Dose dependent effect was also seen in this study. It was found that an increase in concentration (5% w/v, 10% w/v, 15% w/v) of extracts shows higher activity against all the pathogens selected for the study as observed by diameter of Zone. It may be possible that with the increasing concentration of the extract, the higher is the content of secondary metabolites or chemical constituents contained in the extract. Thus, the ability of inhibiting bacterial activity increases. However, sometimes too high concentration of extract can interfere with the bacterial cell wall.

Antimicrobial activity of *Tribulus terrestris* against uropathogens have been reported in earlier studies also. In a study all parts of *Tribulus terrestris* showed antibacterial activity against *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.*[11]

Fruits of *Tribulus terrestris* was found to be having antibacterial activity against clinical isolates of *E. coli* in a separate study.[12] Antimicrobial activity of organic and aqueous extracts from fruits, leaves and roots of *Tribulus terrestris* against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, E.coli.*[13] Al-Bayati FA(2008) reported that methanolic extracts of Tribulus fruits hve microbial activity on ATCC strains of *Streptococcus faecalis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeroginosa* with the concentration of 400, 200, 100, 100 µg / ml in order. In another study, Tribulus fruit extract had a similar or even better effect than some antibiotics used in UTI like Ofloxacilin, Ciprofloxacin, Penicillin G, Gentamycin, Cotrimoxazole, Nalidixic acid and Nitrofurantoin.[14]

Results of this study were in conformity with previous studies. In this study, evaluation was done in both gram positive and gram negative bacteria which are commonly involved in pathogenesis of UTI.

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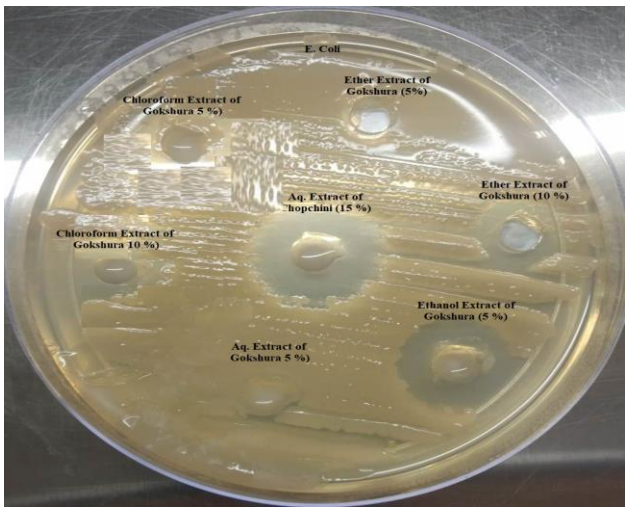

Fig 1: Activity of *Tribulus terrestris* against *E.coli*

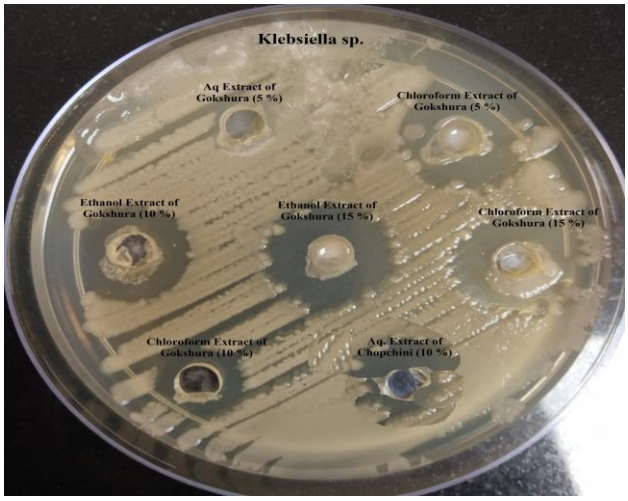

Fig 2: Activity of *Tribulus terrestris* against *Klebsiella pneumoniae*

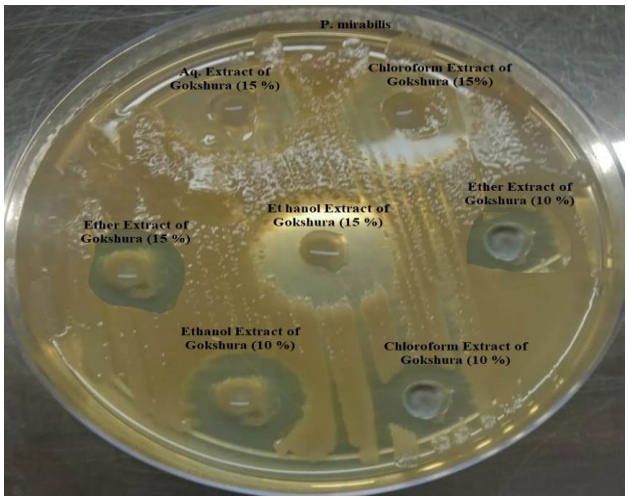

Fig 3: Activity of *Tribulus terrestris* against *Proteus mirabilis*

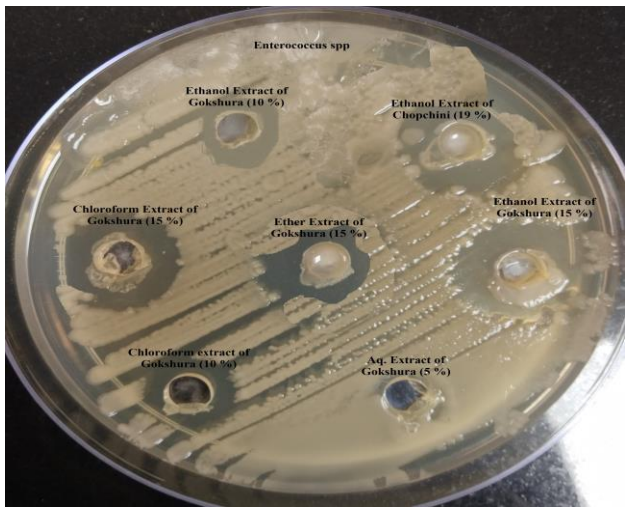

Fig 4: Activity of *Tribulus terrestris* against *Enterococcus faecalis*

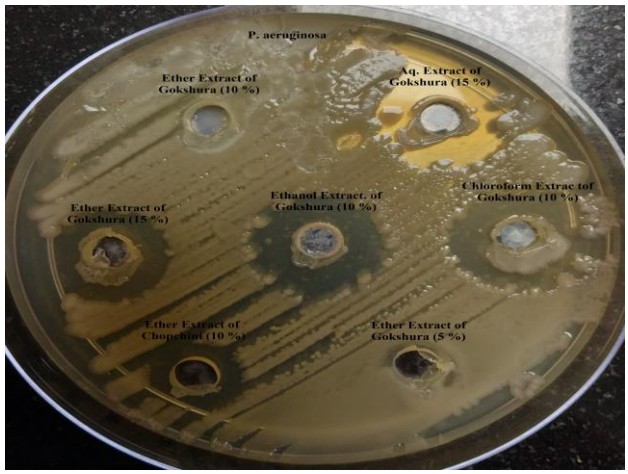

Fig 5: Activity of *Tribulus terrestris* against *Pseudomonas aeruginosa*

CONCLUSION

The results of this study showed that *Tribulus terrestris* possesses significant antibacterial activity against common uropathogens. Therefore, *Tribulus terrestris* must be subjected for further experimental and clinical studies to explore and affirm its efficacy in clinical trials. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index.

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