EVALUATION OF ANTIBACTERIAL ACTIVITY AND MINIMUM INHIBITORY CONCENTRATION OF TRIBULUS TERRESTRIS LEAF EXTRACT AGAINST SENSITIVE STRAINS OF SELECTED HUMAN PATHOGENS

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ABSTRACT: ***The antibacterial effect of Methanolic leaves extract of plant Tribulus terrestris against human pathogenic bacteria namely E.coli ATCC 25922, Klebsiella pneumonia MTCC 3384, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853 were determined using Cup plate method and MIC (Minimum inhibitory concentration) methods. The results in the present study suggests that Present plant extract can be used in treating ailments caused by the tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and due to the presence of various active principles in the leaves on the target organisms.***

Keywords:- Tribulus terrestris, Cup-plate method, MIC.

1. Introduction

Tribulus terrestris belonging to family Zygophyllaceae, commonly known as Gokshur or Gokharu is a hairy shrub founds commonly all over India but chiefly in sandy areas of Gujarat and rajasthan[1, 2]. The plant is traditionally used in folklore as astringent, tonic, urinary disinfectant, palliative, as a diuretics and for the treatment of sexual debility. The whole plant has been explored for its pharmacological activities like antihypertensive[3], antioxidative[4], immunomodulatory[5] and Aphrodisiac[6] etc. Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with anti-microbial activity, with possibly new modes of action. This expectation that some naturally occurring plant compounds can kill antibiotic-resistant strains of bacteria has been confirmed. Unnecessary or questionable use of Antibiotics leads to Resistance, which forces the research community to develop methods of altering structures of antimicrobial compounds to avoid their inactivation. Now these days natural plants and herbal formulations have constructed new research categories for developing new antimicrobial agents[7]. Hence the present study is aimed to assess antibacterial property of plant.

2. Material and Methods.

2.1 Plant Material: Leaves of Tribulus terrestris was collected from the premises of the campus of Rajasthan University, Jaipur. Authenticated by Dept. of botany, Rajasthan University, Jaipur. Whose number is RUBL 211664 and a voucher specimen was deposited in the department for future reference.

2.2 Culture of microorganisms

Pure cultures of all experimental bacteria were obtained from the Microbial Culture collection Division established at CIRD (Centre for innovation, research and development) of Dr. B. L Institute of Biotechnology, Jaipur.

2.3 Extraction Procedure

The collected material was dried in the shade for 5 weeks away from sunlight. The dried material was ground to a coarse powder and extracted (100 g) successively with 600 ml methanol in a Soxhlet extractor at 130^0C for 24-30 hrs. The extract was concentrated by using rotary evaporator to yield a light brown solid. The extract was preserved in a desiccator till further use.
For aqueous extract preparation, 150 grams of coarsely powdered leaves were added to 150 milliliters of boiling distilled water. The resulting mixture was filtered twice on White cotton and once on Whatman filter paper N0-4. The Filtrate obtained is preserved at temperature of 40°C in an oven for drying. The concentrated filtrates were used for further studies at different concentrations[8].

2.4 Preparation of test/stock solution

Test compounds were prepared by taking two different concentrations viz. 50 mg/ml and 100 mg/ml.

2.5. Preparation of Sub-culture:

One day prior to these testing inoculations of the above bacterial cultures were Made in the nutrient agar and incubated at 37˚C to 24 hours.

2.6. Preparation of inoculums:
A loopful of the organisms was emulsified in 100 ml sterile growth media under proper sterile conditions and incubated for 72 hrs at 37˚C.

2.7. Preparation of Agar plates:
The Petri dishes which measures around 90 mm in diameter, 4mm thickness were selected after sterilizing by dry heat in an oven. Base layer was obtained by pouring around 30 ml of Brain Heart Infusion Agar to obtain a approximately Thickness of 4mm(Total 360 ml agar is used for the study). The Petri plates then Kept aside for solidification. Overnight grown sub culture was taken into definite volumes of peptone water and incubated at 37˚C at least for 4 hours prior to plating. After incubation, with the help of cotton swab the organisms were streaked on Petri dish containing base layer medium.

2.8 CUP PLATE METHOD[9, 10]:-
The sterile borer was used to prepare 4 cups of 8mm in diameter. One cup for standard two for 50 mg/ml and 100mg/ml conc. of test compound and one for negative control. All the plates were kept at room temperature for effecting diffusion of the test drug and standard. Later, they were incubated at 37˚C for 24 hours. The presence of definite zones around the cup of any size indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

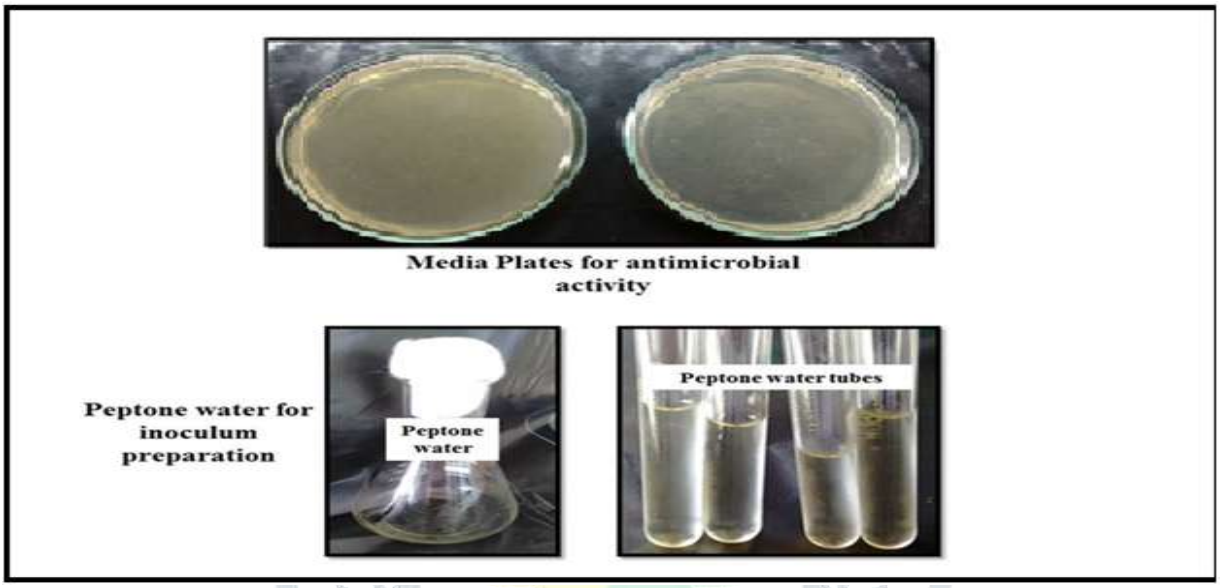


Fig 1: Peptone tubes and Media plates used in the study.

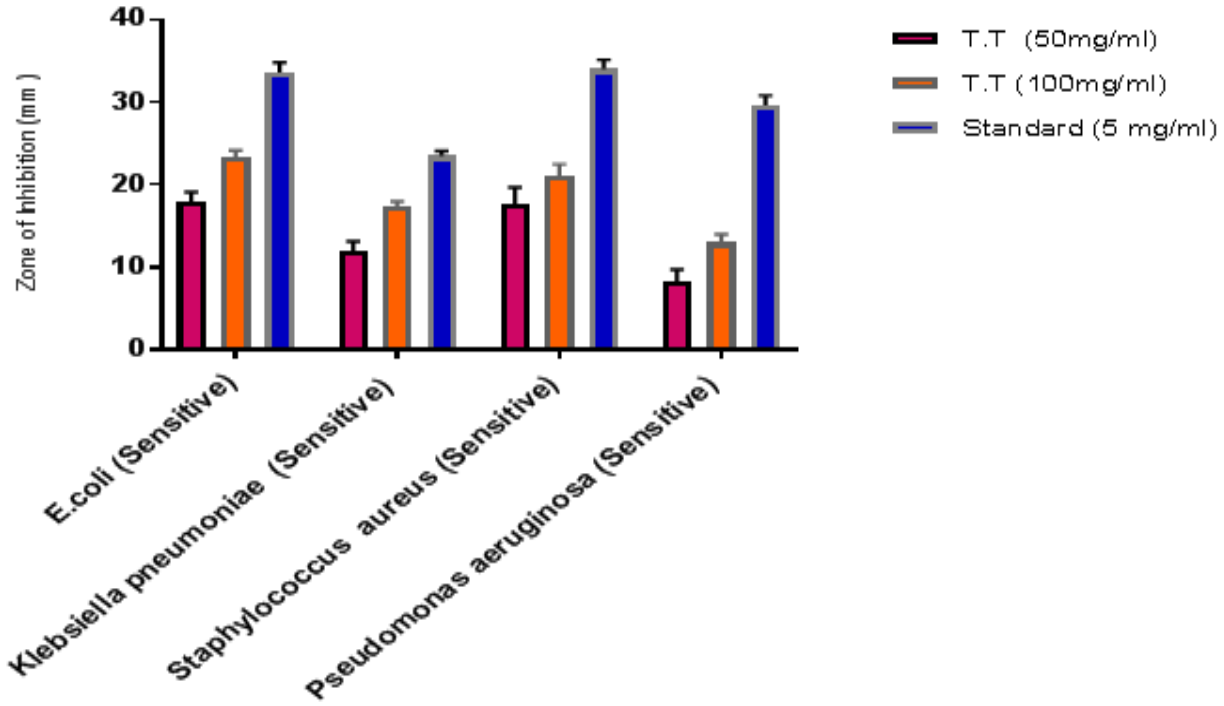
2.9 MIC (MINIMUM INHIBITORY CONCENTRATION) METHOD[11]:
Ten borosilicate sterile glass test tubes were taken and placed in test tube stand. 200 µl of Brain Heart Infusion Broth solution were taken in test tubes serially numbered as 1-10. To the first test tube, 200 µl of stock solution was added; it must be pipette in and out to ensure proper mixing. From the first test tube, 200 µl of mixture was pipette out, mixed in second test tube, like wise from each test tube 200µ l of mixture taken out and added to next test tube in order till the end test tube. After mixing in 10th test tube, 200 µl of solution was pipette out and discarded. The suspension of the organisms of 105 CFU/ml in Brain Heart Infusion Broth was prepared and then 200 µl was inoculated to each test tube from 1st to 10th. Same procedure was followed for all test tubes. All test tubes were incubated at 37˚C for 24 hours, without shaking or agitation. After 24 hours of incubation all test tubes were read for MIC, turbidity indicates that the bacterial growth has not been inhibited by the concentration of the preparation contained in the medium. Total volume in each tube is 400 µl. Final concentration ranges between 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 16 mg/ml, 8 mg/ml, 4 mg/ml, 2.0 mg/ml, 1.0 mg/ml, 0.5 mg/ml, and .25 mg/ml.
Clear solution indicates – Sensitive
Turbidity indicates - - Resistance

Statistical analysis
The values of antimicrobial activity of Tribulus terrestris leaves extracts were expressed as mean ± standard error mean (n= 3) for each sample.

3 RESULTS AND DISCUSSION
The results of antibacterial activity of the Methanolic and aqueous extracts of Tribulus terrestris leaves are presented in Table 1 and 2. The result of the study showed that both extracts had inhibitory effect on the test organisms The Methanolic extract showed highest antibacterial activity against **E. coli ((23.3mm)** followed by **S. aureus (21mm), K. pneumonia (17.3mm)** and **P. aeruginosa (13mm)**. Greater MIC values are indication of low activity while lower MIC values are indication of high activity. In the case of Methanolic extract, E.coli and **Klebsiella pneumoniae** had low MIC values of **4.01 mg/ml** and **8.3 mg/ml** respectively, thus suggesting higher activity against these corresponding organisms. But in the case of aqueous extract Klebsiella pneumoniae and Pseudomonas aeruginosa both shows almost similar Zone of inhibition at the concentration of **100 mg/ml**. However the highest inhibitory zone was observed against **E.coli (17mm)** but the highest MIC value of **4.01 mg/ml** was observed with **Staphylococcus aureus.** These results indicating that the plants could be a good source for the antibacterials to combat bacterial infections caused by tested microorganisms**.**

NAME OF ORGANISM	ZOI (mm)		
	TEST (T.T)		STD
	50mg/ml	100mg/ml	5mg/ml
E.coli (Sensitive)	18±1.15	23.3±0.89	33.6±1.2
Klebsiella pneumoniae (Sensitive)	12±1.15	17.3±.67	23.5±.57
Staphylococcus aureus (Sensitive)	17.67±2.02	21±1.52	34±1.15
Pseudomonas aeruginosa (Sensitive)	8.3±1.45	13±1.0	29.6±1.20

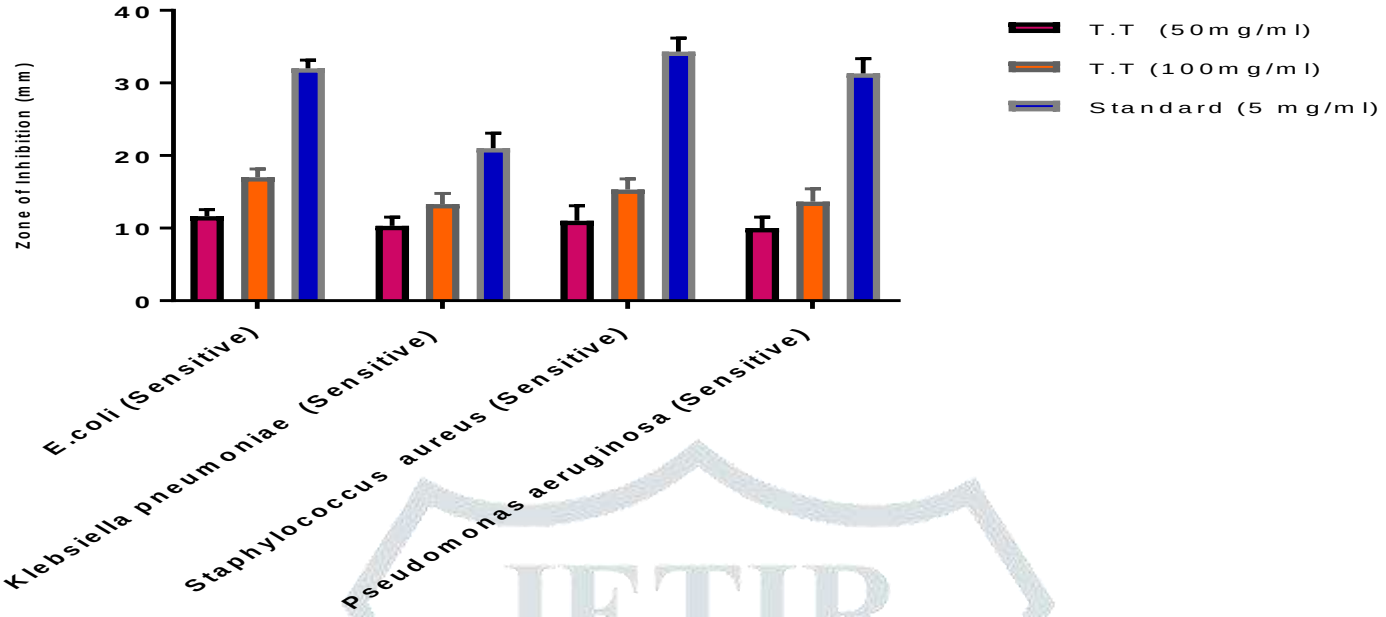
Table1. : - Antimicrobial activity of Methanolic extract of Tribulus terrestris. Values are expressed as mean ± sem, n=3. ZOI*=Zone of Inhibition.



Graph 1. Antimicrobial activity of Methanolic extracts of Tribulus terrestris.

NAME OF ORGANISM	ZOI (mm)		
	TEST (T.T)		STD
	50mg/ml	100mg/ml	5mg/ml
E.coli (Sensitive)	11.66±0.88	17±1.15	32±1.15
Klebsiella pneumoniae (Sensitive)	10.33±1.20	13.33±1.45	21±2.08
Staphylococcus aureus (Sensitive)	11±2.08	15.33±1.45	34.33±1.85
Pseudomonas aeruginosa (Sensitive)	10±1.52	13.66±1.76	31.33±2.02

Table2. : - Antimicrobial activity of Aqueous extract of Tribulus terrestris. Values are expressed as mean ± sem, n=3. ZOI*=Zone of Inhibition.



Graph 2. : - Antimicrobial activity of aqueous extract of Tribulus terrestris.

S.NO	MICROBES	125 mg/ml	62.5 mg/ml	31.25 mg/ml	16.6 mg/ml	8.3 mg/ml	4.01 mg/ml	2.0 mg/ml	1.0 mg/ml	0.5 mg/ml	0.25 mg/ml
1	**E. coli**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**
2	**Klebsiella pneumoniae**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**
3	**Staphylococcus aureus**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**
4	**Pseudomonas aeruginosa**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**

Table 3.: - Evaluatiom of Minimum Inhibitory concentration of Methanolic extract of Tribulus terrestris.

S.NO	MICROBES	125 mg/ml	62.5 mg/ml	31.25 mg/ml	16.6 mg/ml	8.3 mg/ml	4.01 mg/ml	2.0 mg/ml	1.0 mg/ml	0.5 mg/ml	0.25 mg/ml
1	**E. coli**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**
2	**Klebsiella pneumoniae**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**
3	**Staphylococcus aureus**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**
4	**Pseudomonas aeruginosa**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**

Table 4 :-Evaluation of Minimum Inhibitory concentration of Aqueous Extract of Tribulus terrestris.

4.Conclusion:-

This study led to the conclusion that the antimicrobial activity of the Methanolic and aqueous extract of Plant Tribulus terrestris was satisfactory against the bacteria E. coli, Klebsiella pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa. However the Inhibitory zones was extract dependent and Methanol extract was seems to be more effective as compare to aqueous extract, The results are important as a preparation for further research to explore wide regions of naturally obtained antimicrobial agents.

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