



**IN VITRO ANTIBACTERIAL ACTIVITY OF WATER AND ETHANOL EXTRACT OF TRIBULUS TERRESTRIS ON THE GROWTH OF PSEUDOMONAS AERUGINOSA BY DISC DIFFUSION TEST**

Vishal Kumar Deshwal

Department of Microbiology, Doon (P.G.) Paramedical College, Dehradun-248 001, Uttarakhand, India

\*corresponding author and Email ID: vishal\_deshwal@rediffmail.com

Contact number: +919897538555

**ABSTRACT:** The present study was conducted to determine the antibacterial activity of *Tribulus terrestris* against *Pseudomonas aeruginosa*. Four different concentrations i.e. 15mg/ml, 20mg/ml, 25mg/ml, 30mg/ml of different extract of water and ethanol extracts of *Tribulus terrestris* were screened for antibacterial activity by disc diffusion method. Ofloxacin was control drug. Ethanol extract of *Tribulus terrestris* showed maximum inhibition zone i.e. 27.33mm as compared to aqueous extract. Aqueous extract of *Tribulus terrestris* showed inhibition zone from 13.66 to 25.66mm but ethanol extract showed 14.66 to 27.33mm inhibition zone. It was concluded that *Tribulus terrestris* is an effective medicinal plant which shows effective antibacterial activity against *Pseudomonas aeruginosa*.

**Key words:** *Tribulus terrestris*, *Pseudomonas*, antibacterial

## INTRODUCTION

Medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis [1]. Medicinal plants are a source of great medicinal value all over the world [2]. Many plants have medicinal value and recently, Abirami and Rajendran, [3] reported that there are more than 35,000 plants species being used in medicinal purpose. *Tribulus* L. is member of Zygophyllaceae and comprises about 25 species in tropical and warm regions [4, 5]. *Tribulus terrestris*, commonly known as Gokhru. Gokhru (India) is a shrub found in the Southern part and different parts of the plant are used to treat various ailments like, cough, cold and as an antiseptic [6]. Plant is an annual plant distributed in warm regions of Asia, Africa, Europe, America and Australia [7, 8]. *T. terrestris* is mainly known for its effectiveness in libido disorders, impotence and infertility. Data published on its cardiovascular, cytotoxic and antimicrobial activities [9]. *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithon-triptic and urinary anti-infective [10]. Janovská et al [11] observed that results showed that the extracts from *Tribulus terrestris* (aerial part) possessed antimicrobial activity against *Staphylococcus aureus*. Different extract of polar and non-polar solvents of *Tribulus terrestris* showed antibacterial activity against various pathogens [12]. *Pseudomonas aeruginosa* is a major infectious agent that causes pneumonia, otitis media, septicaemia enteritis, sudden death in chinchillas and a leading cause of nosocomial infections in humans [13, 14]. So aim of present study is to evaluate the antimicrobial activity of water and ethanol extract of *Tribulus terrestris* on the growth of *Pseudomonas* by disc diffusion test.

## MATERIALS AND METHODS

**Microorganism:** Characterized *Pseudomonas aeruginosa* strains are taken from Department of Microbiology, DPMC, Dehradun.

**Preparation and selection of different extracts:** Two extracts such as aqueous extract, ethanol extract were selected for present study:

**(a) Preparation of Aqueous extracts:** 100g dried finely powdered of plant were infused in distilled water until completely exhausted. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated and dried using rotary evaporator at 60°C. The final dried samples were stored at low temperature.

**(b) Preparation of Ethanol extracts:** Dried plant was grounded and extracted in a percolator with 95% ethanol. About 10ml of ethanol per gram of sample was used. The ethanol extract was dried under reduced pressure at 40°C. The dried extract was stored in sterile bottles for further use.

**Sterilization and preparation of different concentration of extract:** The dried extracts were exposed to ultra violet light (UV rays) for 24 hours [15]. Liquid extracts were sterilized using a membrane filter (0.2µm sterile filter). Dry powder extracts were initially dissolved in 1ml of dimethyl sulfoxide (DMSO). Different dilutions of extract were prepared. Ofloxacin antibiotic worked as control drug.

**Preparation of disc:** 15mg/ml, 20mg/ml, 25mg/ml, 30mg/ml of different extract as well as control antibiotics was prepared. 20µl of from each extract/antibiotics was used to impregnate a 6mm blank sterilized disc (Hi-media). The final concentration used for the test was from 0.3mg/disc to 0.6mg/disc. These impregnated discs were dried in 37°C incubator for 18 to 24 hours and immediately used for the sensitivity test [16].

**Antibacterial activity of plant extract:** Antibacterial activity was performed according to Deshwal and Vig [17]. The microorganism was activated by inoculating a loopful of the strain in nutrient broth (30ml) and incubated on a rotary shaker. Then 0.2ml of inoculum ( $10^8$  cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the Petri plate. Transferred 6mm disc on the medium and the plates were incubated at 37°C for 24 hours. Microbial growth was determined by measuring the diameter of zone of inhibition. The control zones (solvent) were subtracted from the test zones and the resulting zone diameter is mention in table 1.

## RESULTS AND DISCUSSION

Four various (15mg/ml, 20mg/ml, 25mg/ml and 30mg/ml) concentrations of water extract, ethanol extract of *Tribulus terrestris* and Ofloxacin were used. 20mg/ml water extract of *Tribulus terrestris* gave 6.3% more inhibition zone as compared to Ofloxacin (15mg/ml). 30mg/ml water extract showed 8.4% more inhibition zone as to Ofloxacin (25mg/ml). Similarly, 20mg/ml ethanol extract of *Tribulus terrestris* showed 14.9% more inhibition zone as compared to Ofloxacin (15mg/ml). 30mg/ml ethanol extract of *Tribulus terrestris* showed maximum inhibition zone i.e. 27.33mm as compared to aqueous extract (Table 1). Deshwal and Vig [17] reported the antibacterial activity of *Tribulus terrestris* against *Escherichia coli*. Similarly, ethanol extract of *Cinnamomum cassia* showed maximum antibacterial activity against *Pseudomonas aeruginosa* [18]. Similarly, Clove and Eucalyptus ethanol extracts have great potential antibacterial activity against *P. aeruginosa*, however Hibiscus ethanol extract exhibits more lesser activity as compared to Clove and Eucalyptus [19]. Plants are reported to possess antibacterial activity against certain pathogens which would be useful to clinical uses [17, 20, 21]. The results suggested that *Tribulus terrestris* contains good antibacterial activity which would be useful to control disease caused by *Pseudomonas aeruginosa*.

**Table 1. In vitro antibacterial activity of water and ethanol extracts of *Tribulus terrestris* on the growth of *Pseudomonas aeruginosa* by disc diffusion test**

S.No.	Concentration	Inhibition zone (mm)*		
		water extract	ethanol extract	Ofloxacin
1	15 mg/ml	13.66	14.66	15.66
2	20 mg/ml	16.66	18.00	19.66
3	25 mg/ml	20.33	22.00	23.66
4	30 mg/ml	25.66	27.33	28.66

\*Values are mean of 3 replicate

(15mg/ml=0.3mg/disc; 20mg/ml=0.4mg/disc; 25mg/ml=0.5mg/disc; 30mg/ml=0.6mg/disc)

## REFERENCES

- [1] Sharmin L. 2004. Cultivation prospect of medicinal plants in Bangladesh: experiences from Natore. ([www.bracresearch.org/reports/medicinal\\_plant\\_cultivation.pdf](http://www.bracresearch.org/reports/medicinal_plant_cultivation.pdf)).
- [2] Joshi B, Lekhak S and sharma A. 2009. Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. Kathmandu Univ J Sci, Enginee Technol, 5(1): 143-150.
- [3] Abirami P and Rajendran A. 2011. GC-MS Analysis of *Tribulus terrestris*. L. Asian J Plant Sci and Res, 1 (4): 13-16.
- [4] Boulos L. 2000. Flora of Egypt, Vol. 2 (Geraniaceae – Boraginaceae ). Al Hadara publishing Cairo, Egypt
- [5] Mohamed AH. 2006. Taxonomic Significance of Seed PProteins and Iso- enzymes in *Tribulus* (Zygophyllaceae). Int J Agri Biol, 8(5): 573-575.
- [6] Patel DK, Laloo D, Kumar R and Hemalatha S. 2011. *Pedaliium murex* Linn.: an overview of its phytopharmacological aspects. Asian Pac J Trop Med, 4(9):748-55.
- [7] Topia MO, Giordano MA and Gueper HG. 1994. An Outbreak of Hepato-genous Photosensitization in Sheep Grazing *Tribulus terrestris* in Argentina. Vet Hum Toxicol, 36(4):311-313.
- [8] Abeywickrama K and Bean GA. 1991. Toxigenic *Aspergillus Flavus* and Aflatoxins in Sri Lankan Medicinal Plant Material. Mycopa-thologia, 113: 187-190.
- [9] Kostova I, Dinchev D, Rentsch GH, Dimitrov V and Ivanova A. 2002. Two New Sulfated Furostanol Saponins from *Tribulus terrestris*. Z Naturforsch [C], 57(1-2): 33-38.
- [10] Ody P. 2000. The Complete Guide Medicinal Herbal. London: Dorling Kindersley: 223.
- [11] Janovská D, Kubíková K and Kokoška L. 2003. Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine. Czech J Food Sci, 21: 107-110.
- [12] Hussain AA, Mohammed AA, Ibrahim HH and Abbas AH. 2009. Study the Biological Activities of *Tribulus Terrestris* Extracts. W Acad Sci, Engineer and Technol, 57: 433-435.
- [13] Rorich PJ, Ling GV, Ruby AL, Jang SS and Johnson DL. 1983. In vitro susceptibilities of canine urinary bacteria to selected antimicrobial agents. J Am Veter Med Asso, 8: 863-867.
- [14] Hirakawa Y, Sasaki H, Kawamoto E, Ishikawa H, Matsumoto T, Aoyama N, Kawasumi K and Amao H. 2010. Prevalence and analysis of *Pseudomonas aeruginosa* in chinchillas. BMC Veterinary Research, 6:52 (doi:10.1186/1746-6148-6-52)
- [15] Ekwenye UN and Elegalam NN. 2005. Antibacterial Activity of Ginger (*Zingiber Officinale* Roscoe and Garlic (*Allium Sativum* L.) Extracts on *Escherichia Coli* and *Salmonella typhi*. Int J Mol Med and Adv Sci, 1(4): 411-416.
- [16] Zaidan MRS, Noor Rain A, Badrul AR, Adlin A, Norazah A and Zakiah I. 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Tropical Biomedicine, 22(2): 165-170.
- [17] Deshwal VK and Vig K. 2011. Screening for Antibacterial activity of seeds of *Tribulus terrestris* L. growing in Uttarakhand (INDIA). Int J Pharmaceutical Invent, 1(1): 42-46.
- [18] Sharma A, Chandraker S, Patel VK and Ramteke P. 2009. Antibacterial Activity of Medicinal Plants Against Pathogens causing Complicated Urinary Tract Infections. Ind J Pharm Sci, 71(2): 136-139.
- [19] Kamel GM, Ezz eldeen NA, El-Mishad MY and Ezzat RF. 2011. Susceptibility Pattern of *Pseudomonas aeruginosa* Against Antimicrobial Agents and Some Plant Extracts with Focus on its Prevalence in Different Sources. Global Veterinaria, 6 (1): 61-72.

- [20] Prakash SK. 2006. Effects of herbal extracts towards microbicidal activity against pathogenic *E. coli* in poultry. *Int J Poultry Sci*, 5(3): 259-261.
- [21] Sartorelli P, Marquioreto AD, Amaral-Baroli A, Lima MEL and Moreno PRH. 2006. Chemical composition and antimicrobial activity of the essential oils from two species of *Eucalyptus*. *Phytotherapy Res*, 21(3): 231-233.