

In vitro antibacterial activity of water and ethanol extract of *tribulus terrestris* on the growth of *bacillus* by disc diffusion test

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Abstract

The present study was conducted to determine the antibacterial activity of *Tribulus terrestris* against *Bacillus* which is a potent pathogen whose various species are responsible for causing a number of diseases. Two extracts, namely water and ethanol extracts of *Tribulus terrestris* were screened for antibacterial activity by disc diffusion method, in which filter paper discs of various concentrations of the extract were prepared and used. It was observed that, at a concentration of 30mg/ml both water and ethanol extracts were more effective as they gave larger inhibition zones at this concentration than the other concentrations of 15, 20, 25mg/ml. The present study also showed that the ethanol extract of *Tribulus terrestris* was more effective than the water extract. So, it was concluded that *Tribulus terrestris* is an effective medicinal plant which show effective antibacterial activity against *Bacillus* a potential pathogen.

INTRODUCTION

Medicinal plants are a source of great medicinal value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of country [1]. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal remedies has risen in the developed countries in the last decades [2]. Development of multidrug resistance (MDR) in pathogenic bacteria has created clinical problem. *Bacillus cereus* is ubiquitous in nature, and while most isolates appear to be harmful, some are associated with food-borne illnesses, periodontal diseases, and other more serious infections. There have been reports of severe and sometimes fatal cases of pneumonia caused by *B. cereus* in apparently healthy welders [3, 4].

Tribulus terrestris, commonly known as Gokhru, is a shrub belonging to the Family Zygophyllaceae. Historically, *Tribulus terrestris* was used by the cultures of India and Greece as a rejuvenation tonic [5]. Tilwari et al. [6] reported the *Tribulus terrestris* showed immunomodulating activity. Kianbakht and Jahaniani [2] validated the *in vitro* anti-bacterial activity of methanolic extracts of different parts of *Tribulus terrestris* L. against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa* by broth dilution assay and agar diffusion assay and observed that the methanolic extracts of all parts of the plant showed considerable activity against all bacteria. So aim of present study is to evaluate the antimicrobial activity of water and ethanol extract of *Tribulus terrestris* on the growth of *Bacillus* by disc diffusion test.

MATERIALS AND METHODS

Microorganism: Characterized *Bacillus sp.* strains were taken from Department of Microbiology, Doon (PG) Paramedical College, 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 24h to sterilize [7]. 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 [8].

Antibacterial activity of plant extract: Antibacterial activity was performed according to Deshwal and Vig [9]. 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 (inoculum size was 10⁸ cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the sterilized Petri plate. Transferred 6mm disc on the medium and the plates were incubated at 37°C for 24h. Microbial growth was determined by measuring the diameter of zone of inhibition. The control zones were subtracted from the test zones and the resulting zone diameter is mentioned in table.

RESULTS AND DISCUSSION:

The various concentrations of water extract of *Tribulus*

Table 1: *In vitro* antibacterial activity of water and ethanol extract of *Tribulus terrestris* on the growth of *Bacillus* by disc diffusion method.

S.No.	Concentration	Inhibition zone (mm)*		
		water extract	ethanol extract	Ofloxacin
1	15 mg/ml	15.33	16.33	17.66
2	20 mg/ml	18.66	19.33	22.33
3	25 mg/ml	23.33	24.00	24.66
4	30 mg/ml	25.66	26.00	27.66

*Values are mean of 10 replicate
(15mg/ml=0.3mg/disc; 20mg/ml=0.4mg/disc; 25mg/ml=0.5mg/disc; 30mg/ml=0.6mg/disc)

terrestris that were used are 15mg/ml, 20mg/ml, 25mg/ml and 30mg/ml. Ten strains of *Bacillus* were tested for the antimicrobial activity of *Tribulus terrestris* at the above four concentrations and the mean value of the diameter of inhibition zone (mm) was observed. The result indicated that antimicrobial activity of water extract as well as ethanol extract of medicinal plant *Tribulus terrestris* is less effective as compared to Ofloxacin antibiotic. It was observed that at a concentration of 20mg/ml, water extract of *Tribulus terrestris* gave 5.6% more inhibition zone as compared to Ofloxacin (15mg/ml). At a concentration of 25mg/ml, water extract of *Tribulus terrestris* gave 4.47% more inhibition zone as compared to Ofloxacin (20mg/ml). Similarly, when the comparison was made between the inhibition zones made by the ethanol extracts of medicinal plant *Tribulus terrestris* and Ofloxacin antibiotic, it was observed that at a concentration of 30mg/ml ethanol extract of *Tribulus terrestris* gave 5.4% more inhibition zone as compared to Ofloxacin (25mg/ml) (Table 1). Deshwal and Vig [9] reported the antimicrobial activity of Aqueous, ethanol and chloroform extracts of *Tribulus terrestris* L. against *Escherichia coli*. Further, some researcher also reported the antibacterial activity of medicinal plant [10-12]. Previously, Parekh [13] reported that extract of medicinal plant was effective against medically important bacterial strains. On the basis of present information, we concluded that ethanol extract of *Tribulus terrestris* is much more effective against *Bacillus* as compared to water extract. These result suggested that *Tribulus terrestris* contains good antibacterial properties.

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