



ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT OF *TRIBULUS TERRESTRIS* LINN PLANT

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ABSTRACT

Tribulus terrestris Linn is a herb of the family Zygophyllaceae, whose parts are known to be used as traditional herbal medicine to treat various ailments such as kidney infections. The methanol extract of *Tribulus terrestris* plant was screened for anti-inflammatory and antimicrobial activity. The methanolic extract showed a significant inhibition on the growth of Gram (+) and Gram (-) bacteria at concentrations of 200 µg/mL and 400 µg/mL, respectively. A dose dependent inhibition of rat paw volume by methanolic extract of *T. terrestris* in Carrageenan induced inflammation in rats was observed, which is comparable with standard drug, diclofenac sodium.

Key words : *Tribulus terrestris*, Antimicrobiol, Antiinflammatroy.

INTRODUCTION

Many of the drugs isolated and characterized from plants and extensively used in modern medicine have a folklore origin and are traditionally employed in systems of medicine in curing many ailments¹⁻³. Several drugs and chemotherapeutics have been obtained from naturally occurring products of medicinal plants^{4,5}. *Tribulus terrestris* Linn is a herb, which belongs to the family Zygophyllaceae and is found in waste places and dry habitats throughout the warmer regions of India. The parts of the plant are known to be used as traditional herbal medicine to treat various ailments such as kidney infection,

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impotence, cancer⁶ and fruits of the herb have antihypertensive⁷ activity. The present study was aimed at evaluating the anti-inflammatory and anti microbial activity of methanolic extract of *T. terrestris* (METT) plant.

EXPERIMENTAL

Materials and Methods

Plant material collection

The whole plant material of *Tribulus terrestris* was collected from Andhra University Campus, Visakhapatnam, India. It was authenticated by Dr. M. Venkaiah, Associate Professor of Botany, Andhra University, Visakhapatnam, India.

Extract preparation

The whole plant was dried under shade and then powdered. The powdered material was subjected to continuous hot extraction process using a Soxhlet extractor. Methanol was used as a solvent for extraction and this process was continued until the solvent extraction becomes colorless. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure at 50-55°C and stored in vacuum desiccator. The suspension of the extract prepared in 2% gum acacia was used in the entire experimental studies.

Phytochemical screening

The methanolic extract was screened for the presence of various phyto-constituents like steroids, alkaloids, terpenoids, glycosides, flavonoids and carbohydrates⁸.

Animals

Male wistar rats (175-250 g) were obtained from Mahaveer Enterprises, Hyderabad. The selected animals were housed five per each of acrylic cages at 25°C, 45-55% humidity and 12/12 h light/dark under controlled environment. Animals were fed with standard laboratory diet and water was given *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session.

The animals were divided into different groups, each consist of six animals, were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee.

Screening for anti-inflammatory activity by rat paw edema method

The normal paw volumes of all the rats were measured initially and were divided into four groups, each consists of six animals treated orally with the vehicle as control (2% gum acacia), standard diclofenac sodium (20 mg/kg, p. o.) and methanolic extract (200 and 400 mg/kg), respectively. Carrageenan (0.1 mL of a 1% suspension in saline was injected sub plantar region of the right hind paw of each rat. The vehicle, drug and extract were administered 30 min prior to the injection of carrageenan. The paw volumes of all the rats were recorded at 1, 2 and 4 hr after carrageenan administration using plethysmometer⁹. A significant reduction in the paw volume compared to vehicle treated control animals was considered an inflammatory response.

$$\% \text{ Inhibition} = [(V_T - V_0) \text{ control} - (V_T - V_0) \text{ treated groups}] / (V_T - V_0) \text{ control} \times 100$$

V_0 = Paw volume of the rat before administration of carrageenan

V_T = Paw volume of the rat after administration of carrageenan at different time intervals

Screening for antimicrobial activity using cup plate method

The methanolic extract of *Tribulus terrestris* was evaluated for antimicrobial activity using cup plate method against gram +ve organisms like *Bacillus subtilis*; *Staphylococcus aureus* and gram -ve organisms like *E. coli*; *Proteus vulgaris*. The cup plate technique described by Hugo and Russel¹⁰ was adopted for anti-bacterial activity. Specified quantity of beef extract, peptone and agar were accurately weighed, dissolved in distilled water and sterilized by autoclaving at 121°C for 15 minutes. The plates were prepared with the assay media was cooled to 50°C. It was then inoculated with the test organisms. Four bores per plate were made using sterile cork borer. The above operation was carried out under aseptic condition in sterile area. 0.1 mL of the test compounds, standard and control were incubated at 37°C for 18-24 hours. At the end of 24th hour, the zone of inhibition produced by the methanolic extract at 100 was measured using a scale. The zone of inhibition obtained by different doses of extract was compared with that of standards.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the methanolic extract of *Tribulus terrestris* (METT) reveals the presence of steroids, terpenoids, carbohydrates and glycosides.

Anti-inflammatory activity

Carrageenan induced paw edema test provides a skin inflammation model suitable for evaluation of topical and systemic anti-inflammatory agents. The results of METT against carrageenan induced paw edema is shown in Table 1.

There was a dose dependent inhibitory activity in carrageenan induced paw inflammation at all assessment times. Diclofenac sodium, a COX-inhibitor significantly reduced the paw edema at the dose of 20 mg/kg. This indicates action against release of histamine, serotonin and kinins in early phase, while later phases are suspected to be arachidonate metabolites producing an edema dependent on mobilization of neutrophils¹¹.

Table 1 : Effect of methanolic extract from *Tribulus terrestris* on the paw edema test in rats

Group	Dose (mg/kg)	Paw edema volume (mL)			
		1 hr	2 hr	3 hr	4 hr
Control		0.19 ± 0.03	0.21 ± 0.04	0.22 ± 0.03	0.23 ± 0.02
Diclofenac Sod	20	0.14 ± 0.01 ^a	0.13 ± 0.02 ^c	0.12 ± 0.02 ^c	0.11 ± 0.02 ^c
<i>T. terrestris</i>	200	0.16 ± 0.03 ^a	0.15 ± 0.02 ^b	0.14 ± 0.03 ^c	0.12 ± 0.02 ^a
<i>T. terrestris</i>	400	0.15 ± 0.02 ^a	0.14 ± 0.03 ^c	0.13 ± 0.02 ^c	0.11 ± 0.03 ^a

Values are in mean ± SD; (n = 6), a = p < 0.05, b = p < 0.001, c = p < 0.005 vs. Control

Antimicrobial activity

Many workers have reported on the antibacterial activity of natural products obtained from various sources of plant materials such as seeds³, roots¹, plant parts² etc. Great attention is directed towards isolation, identification and synthesis of the natural products active against a wide variety of bacteria and fungi that cause diseases both in humans and animals. In this direction, the antimicrobial activity of methanolic extract of *Tribulus terrestris* herb was tested against four species of bacteria namely gram +ve organisms like *Bacillus subtilis*; *Staphylococcus aureus* and gram -ve organisms like

E. coli, *Proteus vulgaris*. The extract showed significant dose dependent antimicrobial activity against the tested organisms and results were shown in the Table 2. The technique used was agar diffusion method using 100 µg / 0.1 mL of amoxicillin and gentamicin as standard (Table 2). However, the zone of inhibition exhibited by the test extracts was found to be less than that of their respective reference standard drugs.

Table 2 : Antimicrobial activity of methanolic extract of tribulus terrestris

Compound	Zone of inhibition (mm)			
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. vulgaris</i>
Amoxicillin	28	30	34	29
Gentamicin	25	27	32	28
<i>T. terrestris</i> (200µg/mL)	17	18	20	20
<i>T. terrestris</i> (400µg/mL)	19	21	23	22

In conclusion, the results concluded that methanolic extract of *Tribulus terrestris* plant might have antimicrobial and anti-inflammatory activities. Further studies are necessary to elucidate the mechanisms behind its traditional effects.

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