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Research

Phytochemical Screening and Antimicrobial Activity of Aqueous and Ethanolic Extracts of *Tribulus terrestris* (Zygophyllaceae)

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Abstract

This study was carried out with an objective to investigate the antibacterial and antifungal potentials of leaves of *Tribulus terrestris*. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the bacterial activity of aqueous and ethanol extracts of leaves of *Tribulus terrestris*. The extracts was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal properties of *Tribulus terrestris* extracts (100, 200 µg/ml) were evaluated against two human pathogenic bacteria strains: one fungal strain, *Candida albicans* and one Gram-positive, *Streptococcus pyogenes* and one Gram-negative, *Klebsiella pneumoniae*. The extracts' zones of inhibition were compared to those of other standards, such as the antibacterial activity of amikacin and the antifungal activity of ketoconazole. The outcomes demonstrated a striking reduction of bacterial growth when seen against the species under test. The plants' phytochemical analyses were completed. *Tribulus terrestris* exhibites microbial activity as a result of the existence of several secondary metabolites. Therefore, it is possible to find bioactive natural compounds from these plants that could lead to the establishment of new pharmaceutical research initiatives.

Keywords: *Tribulus terrestris*, Antibacterial activity, Antifungal activity, Antioxidant

INTRODUCTION

The Zygophyllaceae family includes the genus Tribulus, which has approximately 20 species worldwide. Three of these species are commonly found in India: *Tribulus cistoides*, *Tribulus terrestris* and *Tribulus alatus* [(1)]. Not only are modern herbalists and Ayurvedic seers found of *Tribulus terrestris* (TT), but it is also a highly-prized

therapeutic herb [2]. Both as a primary and secondary ingredient in several chemical formulations and dietary supplements or as a stand-alone medicinal treatment, the plant is utilized extensively. This shrub grows as an annual in areas with desert, Mediterranean and subtropical climates, such as India, China, southern USA, Mexico, Spain and Bulgaria [3,4].

Taxonomical classification
Kingdom: Plantae
Division: Phanerogams
Subdivision: Angiospermae
Class: Dicotyledonae
Subclass: Polypetalae
Series: Disciflorae
Order: Giraniales
Family: Zygophyllaceae
Genus: *Tribulus*
Species: *terrestris Linn*.

Fig 1: Plant of *Tribulus terrestris*

Habitat

The natural habitats of *T. terrestris* are warm temperate and tropical parts of southern Europe, western and southern Asia, all of Africa, and Australia. Geographically, *T. terrestris* is found throughout Africa, Southern Europe, China, Japan, Korea and Western Asia [5, 6]. Although T. *terrestris* grows well in soils with light texture, it can tolerate a variety of soil types. It usually grows in lawns, overgrazed pastures, roadsides, farmed crops and neglected places.

Botanical description

T. terrestris is a tiny, prostrate herb with hairy, silky stems that can grow up to 2 meters in length, according to botany (Fig. 1). Each of the pinnate, opposing, short (~1.25 cm) leaves has four to eight pairs of spear-shaped leaflets on it. *T. terrestris* is distinguished by prickly fruits and tiny, yellow, petal-shaped blooms (8–15 mm in diameter). The fruits are woody burrs that have a diameter of around 1 cm and sharp spines that can reach up to 6 mm. Five wedge-shaped segments make up a burr. There are two unequal pairs of spines in each section. Carpels, which are woody structures with a star-like shape that measure approximately 5-7 mm in length and 5-6 mm in breadth, enclose seeds. Each carpel can contain up to five seeds, each of which is 1.5–3 mm long and yellow in hue. A plant is capable of producing up to 2000 seeds. They have a pleasant, astringent flavour. When the root is young, it is light brown in colour, thin and fibrous. The stomata of *T. terrestris* are anomocytic in which the guard cells are not surrounded with any subsidiary cell [7].

Traditional Uses

Tribulus terrestris is a tonic, aphrodisiac, palliative, stomachic, astringent, diuretic, lithotriptic, antihypertensive and urinary disinfectant in traditional medicine. For the majority of genitourinary tract conditions, the herb's dried fruit is particularly helpful. It is an essential part of Gokshuradi Guggul, a powerful Ayurvedic remedy that helps the genitourinary system work normally and gets rid of urinary stones. *Tribulus terrestris* has been used for millennia in Ayurvedic medicine to treat sexually transmitted infections, impotence, and debility. The herb is used as a traditional remedy in Bulgaria to alleviate impotence. The Indian Ayurvedic Pharmacopoeia describes the fruit and root as having cardiotonic qualities in addition to all these other uses. The

fruits were used to cure a variety of conditions in traditional Chinese medicine, including edema, emission, morbid leukorrhea, stomach distension and sexual dysfunction. According to the Shern-Nong Pharmacopoeia, the earliest known pharmacological work in China, *Tribulus terrestris* is a very valuable medication that can help restore the depressed liver and treat vitiligo, mastitis, flatulence, acute conjunctivitis, headaches and chest fullness. *Tribulus terrestris* is a diuretic, mild laxative and general tonic in Unani medicine[8].

MATERIALS AND METHODS

Collection and authentication

The Aerial plant of *Tribulus terrestris* was collected from cricket ground of Madurai Medical College, Madurai. It was identified and authenticated by DR.D.Stephen, Assistant Professor, Department of Botany, The American College, Madurai-20,

Extraction Process

Pre-Extraction Preparation of Tribulus terrestris

The aerial plant of *Tribulus terrestris* were collected as whole plant and some foreign matter are removed from the plant followed by washing .The whole plant was dried for about 10 days under shade away from sunlight. The dried plant was grinded to powder. Now the powder is subjected to extraction.

Preparation of plant extract

Aqueous extraction

Fine shoot powder was dissolved in 10% (w/v) sterile distilled water separately in an Erlenmeyer flask to prepare the aqueous extract. The flasks were placed on an orbital shaker for 24 h to allow extraction and then evaporated using a rotary evaporator at 60°C. [9] The final dried samples were stored in labeled sterile bottles and kept at 4°C.

Ethanol extraction

Plants were dried for 2 weeks at room temperature, pulverized to a powder, and passed through a No. 40 sieve. The plant powder (100 g) was weighed, transferred to a round-bottom flask and treated with 95% ethanol using a Soxhlet apparatus. The process lasted 24 h and was maintained at 45–47°C. The extract was then collected and evaporated using a vacuum distillation unit and stored at"20°C.

Phytochemical screening

Qualitative phytochemical screening of T. *terrestris,* was performed to determine the presence of biologically active compounds or secondary metabolites, including saponins, alkaloids, phenolic, flavonoids, carbohydrates, proteins, amino acids, glycosides, tannins and terpenoids [10]. The ethanolic extract was subjected to phytochemical screening and Thin Layer Chromatography analysis of ethanolic and aqueous extract of *T.terrestris* [11, 12, 13].

Test microorganisms and growth media

The following microorganisms *streptococcus pyogenes* (MTCC 442), *Candida albicans* (MTCC 227) were chosen based on their clinical and pharmacological importance [14] .The bacterial strains was used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium, respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C). The stock cultures were maintained at 4°C.

Antimicrobial Activity

Determination of zone of inhibition method

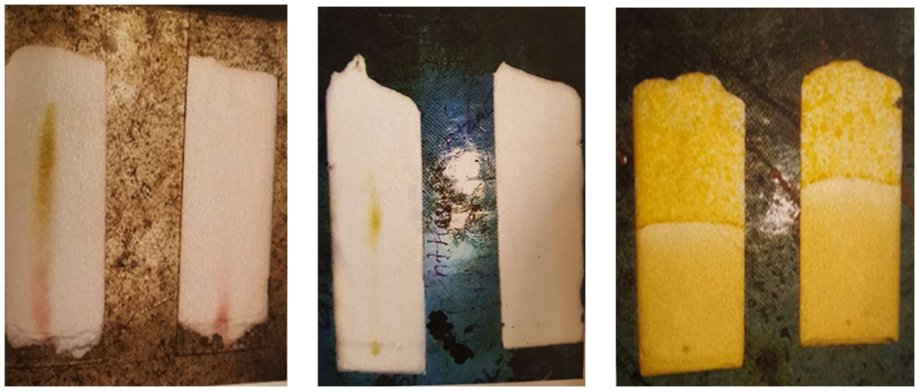
In vitro antibacterial and antifungal activities were examined for Aqueous and ethanolic extracts. Antibacterial and antifungal activities of plant part extracts against two pathogenic bacteria (two Gram-positive and negative) and one pathogenic fungi were investigated by the agar disk diffusion method [15, 16, 17].Antimicrobial activity testing was carried out by using agar cup method. For the determination of zone of inhibition, pure Gram-positive, Gram-negative and fungal strains were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antibacterial and antifungal activities against the *Streptococcus pyogenes, Klebisella pneumoniae* and the fungi *Candida albicans,*The sets of two dilutions (100, and 200 µg/ml) of Tribulus terrestris extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using Amikacin for antibacterial activity ketoconazole for antifungal activity as standard drugs. The zones of growth

inhibition around the disks were measured after 48 hours of in incubation at 37°C for bacteria and 7 days for fungi at 28°C.The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of ethanolic and aqueous extracts of *Tribulus terrestris*

S.NO	Phytochemical Constituents	Result	
		Ethanolic Extract	Aqueous Extract
1	Carbohydrates	+	+
2	Proteins and amino acids	+	+
3	Flavonoids	+	+
4	Glycosides	+	-
5	Phenols	+	-
6	Tannins	+	+
7	Saponins	-	+
8	Alkaloids	+	-
9	Terpenoids	+	+
10	Steroids	+	+
	(+)-Present	(-)- Absent	



Amino acid **Glycoside** **Alkaloid**

Fig 2: TLC of ethanolic and aqueous extracts of *Tribulus terrestris*

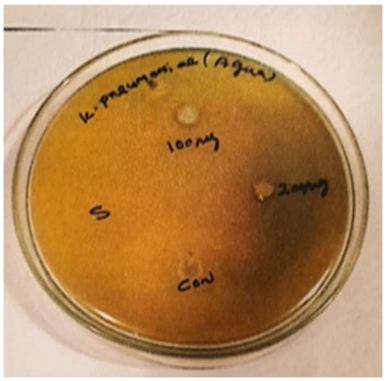
Table 2: Thin layer chromatography of *Tribulus terrestris*

S.No	Chemical Constituents	Aqueous Extract	Ethanolic Extract	Apperance Of Spot
1	Alkaloid	Absent	Present	Yellow
2	Aminoacid	Present	Present	Purple
3	Glycosides	Absent	Present	Green

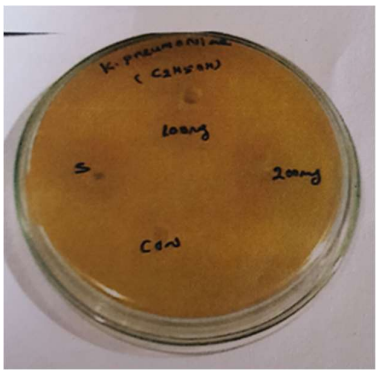
Aqueous extract of *T.terrestris*

Ethanolic extract of *T.terrestris*

Fig 3: Anti bacterial activity of *Staphylococcus pyogenes*



Aqueous extract of *T.terrestris*

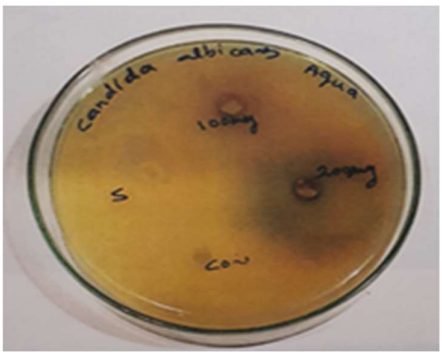


Ethanolic extract of *T.terrestris*

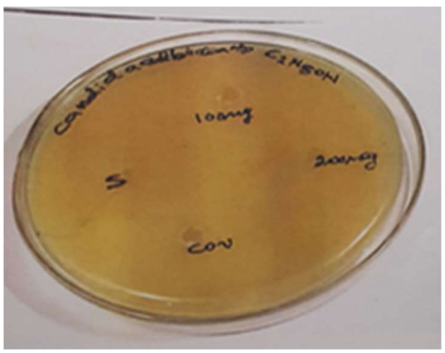
Fig 4: Antibacterial activity of *Klebisella pneumoniae*

Table 3: Antimicrobial activity against Pathogenic Microorganism

Sample		Zone Of Inhibiton		
		Antibacterial Action		Antifungal Action
		Staphylococcus pyogenes	*Klebisella pneumonia*	*Candida albicans*
Ethanolic extract	100 µg/ml	10mm	10mm	11mm
	200 µg/ml	10mm	10mm	11mm
Aqueous extract	100 µg/ml	Resistant	Resistant	Resistant
	200 µg/ml	Resistant	Resistant	Resistant
Control		Resistant	Resistant	Resistant
Standard		15mm	15mm	14mm



Aqueous extract of *T.terrestris*



Ethanolic extract of *T.terrestris*

Fig 5: Anti-fungal activity of *Candida albicans*

From the above result obtained, the highest activity was obtained with ethanolic extract of *Tribulus terrestris*, Which showed broad spectrum anti bacterial activity against gram-positive, gram negative and candida strains. Specifically, these extract produce inhibition zone of 10-15 mm. The aqueous extract of Tribulus terrestris shows resistance against gram positive ,gram negative and candida strains.

CONCULSION

This study showed that ethanolic and aqueous extracts of Tribulus terrestris have the potential to inhibit the growth of various gram-negative and gram-positive bacterial species, and fungi. Further studies are necessary to elucidate the mechanisms behind its traditional effects.

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