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RESEARCH ARTICLE

STUDY OF ANTIBACTERIAL EFFECT OF *TRIBULUS TERRESTRIS* EXTRACT.

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Key words:-
Tribulus terrestris, antibacterial.

Abstract

This study was designed to evaluated antimicrobial activity of *Tribulus terrestris* aqueous extract against some pathogenic microorganisms. So that, the aqueous extract of *Tribulus terrestris* was screened for its anti-microbial activity using the plate agar diffusion method. It was tested against four bacteria species; two Gram-positive bacteria (*Bacillus subtilis*, and *Staphylococcus aureus)* and two Gram-negative bacteria *(Escherichia coli and Pseudomonas aeruginosa).* The susceptibility of these different bacterial species toward the extracts of this plant was compared with each other and with selected antibiotic (streptomycin) used as positive control. Result showed that aqueous extract of *Tribulus terrestris* had antibacterial

Introduction:-

The genus *Tribulus terrestris* (Zygophyllaceae) comprises with 20 species which grow as shrubs in subtropical areas around the world and only two species distributed in China, *T. terrestris* and *T. cistoides*. In traditional Chinese medicine, the fruit of *T. terrestris*, which is known as "Ci Ji Li", has been used against diverse diseases for a long time. The crude saponin fraction of the whole plant has been used as a convivial drug *Tribulus terrestris* (L.,) is also known as puncture vine or small caltrops 10 to 60 cm height, annual herb, with pinnate leaves and yellow flowers . Its carpel fruits are very distinguishing in nature and are known as "Chih-hsing" in China or "Goat head" in the USA. The plant can be found in arid climate regions around the world as in southern USA, Mexico, Spain , Bulgaria, India, and China (Mahammad *et al*., 2012).

Taxonomy of the Plant
Class Dicotyledons
Sub class Polypetatae
Series Thalamiflorae
Order Geraniales
Family Zygophyllaceae
Genus *Tribulus*
Species *terrestris*8

Materials and Methods:-
Plant material: Purchase from local market.
Method of extraction: A quantity of 50 g of the flower powder was mixed with 250 ml sterile double distilled water. The mixture was left in a shaker incubator at 50^{O}C for 24 hours, than filtered through a filter paper

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(Whatman No. 1). The filtrate was concentrated using rotary evaporator at 40^{o}C until dryness and the extract residue was weighted and kept until use (Zheng *et al*., 1990).

Phytochemical evaluation*:* Phytochemical examinations were carried out for all the extracts as per the standard methods (Su *et al*., 2012). Detection of alkaloids, flavonoids, saponins, tannins, trepans and steroids.

Preparation of Tested Microorganisms: The average number of viable *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* microorganisms per ml of the stock suspensions was determined by means of the surface viable counting technique (Chakraborthy *et al*., 2011). About (10^{8}-10^{9}) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that bacterial suspensions with closed viable counts would be obtained.

In Vitro Testing Anti-Microbial Activity of extract: The cup-plate agar diffusion method was adopted according to the method described elsewhere in order to assess the antibacterial activity of the prepared extracts. An amount of 0.6ml of standardized bacterial stock suspensions (10^{8}-10^{9}) colony- forming units per ml was aliquot of mixed with 60ml of sterile nutrient agar. About 20ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set in each of these plates. Bores were made on the medium using sterile borer and 0.1 ml of the extracts were added to bore at conc.20mg/ml and 0.1ml of the standard Streptomycin at a concentration of 100 µg / ml was taken as standard The plates were then incubated in the upright position at 37ºC for 18 hrs. Two replicates were carried out for the extract against each of the tested organism. After incubation, the diameters of growth inhibition zones were measured (Toshiyuki *et al*., 2001).

Results:-

Phytochemical investigation of aqueous extracts of *Tribulus terrestris. Tribulus terrestris* exhibited presence of saponins, terpenes, tannins and flavanoids in its extract. (Table 1).

Table 1:- Aqueous active compounds in *Tribulus terrestris* extract

No.	**Active compounds**	**Aqueous extract of *T. terrestris***
1. 1:	Tannins	_
2. 2:	Saponins	+
3. 6:	Alkaloids	+
4. 7:	Flavonoids	+
5. 9:	Terpenes	+
6. 10	Steroid	+

The symbol (+) refers to presence of compound and the symbol (−) refers to absence of cmpound.

Antibacterial activity of *Tribulus terrestris* extract against the tested organisms are as follows: In case of *Tribulus terrestris*, aqueous extracts inhibited the growth of the bacteria used in the study. (Table.2) and (figure.1)

Table 2:- Antibacterial activity of *Tribulus terrestris* against some pathogenic bacterial species as compared with streptomycin.

Drug	**Mean Diameter of Growth Inhibition Zone in (mm).**			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
Tribulus terrestris	17	15	0	0
streptomycin	35	25	20	0

Discussion:-

Tribulus contains biologically active substances as steroids, saponins, flavonoids, alkaloids. Glycoconjugates are a class of complex molecules that are widely distributed in the plant kingdom and in some marine organisms. This class of compounds has a wide range of biological activates as anti-inflammatory, antimicrobial, antifungal, anticancer and other benefits. Among these compounds, steroidal and triterpenoid saponins have long been known as components of widely used herbal drugs and pharmaceutical preparation (Javed *et al.*, 2012).

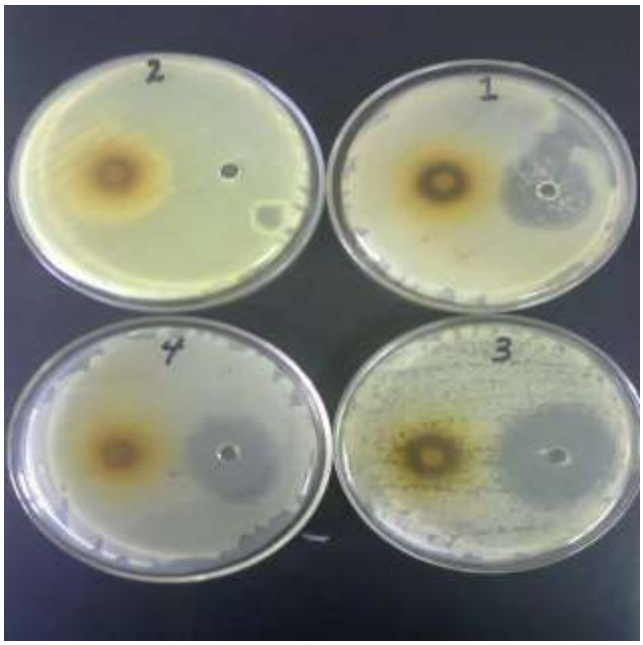

Figure.1:- Antibacterial activity of of *Tribulus terrestris* extract against compared with streptomycin (1) *Staphylococcus aureus, (2) Pseudomonas aeruginosa, (3) Bacillus subtilis, and (4) E. coli.*

The saponins are distinguished as steroidal saponins and triterpenoid. The steroidal saponins include the following chemicals: terrestrosin, diosin, gracillin, kikuba, PTN, nohecogenin, glucoside, tribulosi and F-gitonis (Lee.,2012).The other constituents are flavonoids which include the following substance: Kaempferol, kaempferol glycosides and quercertin. The other minor constituents are present like fatty acids (palmitic, stearic, oleic and linoleic) acids (Kaul *et al.*, 2012).

Tribulus also contains small amounts of alkaloid, tannin, potassium salts, cinnamic amide, resine and sugar. It also presents fixed and essential oils, porphyrin, saprogenic ,25 species of flavonoids-glycosides and resin . Isolation of four furostanol saponins are known as: methylprotodioscin, protodioscin, methylprototribestin and prototribestin. The quality of this herb is dependent on the PTN content (Mona *et al*., 2014).

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