Selva Kumar S. *et al.* / *International Journal of Phytopharmacology.* 3(2), 2012, 91-94.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

STUDY OF PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *TRIBULUS SUBRAMANYAMII* L.

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ABSTRACT

Tribulus species is among the medicinal plants that are used by south Indian traditional practitioners for the aphrodisiac, diuretic effect, reduce inflammation, purify the blood, stones in the bladder, cure skin and heart disease. However, the claims of therapeutic success of medicinal plants by traditional medicine practitioners are hardly subjected to scientific scrutiny. Our study, based on evaluation of the in-vitro antimicrobial activity of ether, chloroform and ethanol extracts of *Tribulus subramanyamii* Linn against standards bacterial and fungal strains by using disc diffusion method. The MIC value of extracts against gram positive bacteria's were ranging between 300 to 500 µg, gram negative bacteria were >600µg and all extracts were ineffective against fungi. The data obtained from the phytochemical analysis of the leaves of T.Subramanyamii indicated the presences of carbohydrates, steroids and glycosides.

Keywords :- Tribulus species, *Tribulus subramanyamii*, Zygophyllaceae, Antimicrobial, Active constituents.

INTRODUCTION

India is known as the "Emporium of Medicinal plants" due to occurrence of several thousands of medicinal plants in the different bioclimatic zones. Ayurveda and Sidddha system of medicine. The traditional heritage of India include many time tested medicinal plants and drugs for various diseases. The demand for Ayurvedic, herbal drugs and phytomedicines is increasing day by day globally. Most cultures from ancient times to the present day have used plants as a source of medicine. A considerable percentage of the people in both developed and developing countries use medicinal plant remedies. In the developed countries consumer are seeking visible alternatives to modern medicine due to its dangers of over medication consequently there is growing interest in medicinal plants and traditional medicine. This immense surge of public interest in the use of plant as medicine has been based on the assumption that the plants will be available on a continuing basis. However in recent years the introduction of new synthetic pharmaceuticals has out placed that of natural products. Many natural products are used in pharmaceutical in their chemical structure; successful efforts have been made to improve their pharmaceutics and therapeutic properties by structural modifications (Showkat Ahmed G *et al*., 2011). The earliest drugs discoveries were made by the presumably random sampling of higher plants. Herbal remedies have been

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valuable drugs for centuries. Knowledge of crude drugs was codified in to the discipline of pharmacogonosy, which has held a distinguished place in the pharmaceutical sciences. The survey shows that the fruits of tribulus species have been used in traditional medicine for the treatment of edema, abdominal distention, and sexual dysfunction (Walid H.EI-Tantawy *et al*., 2007). Roots and fruits are useful in rheumatism, piles, renal vesicle calculi, menorrhagia, anti-oxidant (Kardy., 2010), general weakness, improve appetite, reduce inflammation (Shah *et al*., 2011), cure skin and heart disease. The fruits flowers and leaves reported to possess diuretic, antimicrobial, hemolytic activity (Christo C*et al*., 2011), purity the blood, aphrodisiac (Gauthaman *et al*., 2002 & 2008), hyper-cholesterolemia, vasodilator (Phillips *et al*., 2006), smooth muscle spasms and colic pains. The seeds are have wide range of biological properties such as cooling, diuretic, aphrodisiac (Setiawan *et al*., 1996), remove inflammations, urinary tubes, stones in the bladder, urinary stones, urinary antiseptic (Akram *et al*., 2011). This observation prompted us to study the phytochemical constituents and evaluate the antimicrobial activity of leaf extracts of *Tribulus subramanyamii*.

PLANT DESCRIPTION

Tribulus subramanyamii is found to be growing in sub topical areas around the south India, belonging to the family Zygophyllaceae (Amaal Hasan Mohamed., 2008). It is a tap rooted herbaceous perennial plant that grows as a summer annual in colder climates. Prostrate, spreading herb, branches up to 40cm long, bulbous-based. Leaves are usually opposite one of each pair alternately smaller or aborting, sometimes lower ones alternate (2.0) 3.5-4.5 (6.0) cm long. Leaflets usually 5 pairs, rarely 3,4or 6 pairs, terminal pair always directed upward, sub sessile, ovate or ovate – oblong (6.0) 8.0-15.0 (-17.0) ×(4.0-) 6.0 – 9.0 (-10.0) m unequal sided, apex apiculate or mucronate, lateral nerves in conspicuous. Flowers are only small, 8-15 mm in diameter and bright yellow in colour and have 5 petals, 3-3.5 mm long. The flowers only last one day. Fruit consists of a woody burr with sharp rigid spines, which splits in to segments when ripe. Petals, free, yellow, oblanceolate, 6.0-8.0 × 3.5-5.0 mm apex undulate or emariginate, finely veined, membranous, glabrous, deciduous. Stamens usually 10.5 opposite to each petal and attached at a base rarely only 5 ,filaments fill form 4.0 – 5.0 mm long, glabrous anthers oblong–cordate 1.0- 1.5 mm long. Ovary cyclinderic densely covered with sericeous or hirsute upward spreading bulbous based hairs 2.0 -2.5mm long. 5-locuted; style 1.5 -2.5 mm long, densely brownish pubescent, stigma pyramidal 5-rigid papillose (Vaaghese. M. *et al*., 2006).

PHYTOCHEMICAL CONSTITUENTS

The literature survey indicated that the following phytochemical constituents are present in the tribulus species are disogenin, gitogenin, kaem ferol, glycosides, chlorogenin, tribulosite, disogenin, trilin, tribulusin, tribulosaponin A (Kostova *et al*., 2001), tribulosaponin B, isoherrestrosin B, tribulosaponin A,tribulosaponin B,ishoerrestrosin B,jasmonic ,ruscogenin, tigogenin, kaempferol, Iso rhamnetin-3-rutinosly-4-rhamnoside and neotrigogenine (Simeonov *et al*., 2011).

MATERIALS AND METHODS

EXTRACTION

Air dried coarse powder plant material (3.5kg**)** was defatted with petroleum ether. The marc left after the petroleum ether extract was dried and extracted with diethyl ether at for 36hrs (40°c) by continuous hot percolation using soxlet apparatus. The extract dried by evaporation, a green residue was obtained. The marc left after that extracted with chloroform (50°c-60°c). The extraction was continued up to 48 hours. The chloroform extract was filtered and concentrated by vacuum distillation. The dark green residue was obtained. The marc was extracted with ethanol. The extraction was continued up to 72 hours (75°c-80°c). The ethanol extract was filtered and concentrated by vacuum distillation. A greenish yellow residue was obtained. The extracts were tested for qualitative analysis for the following phytochemical constituents.

AGAR DIFFUSION ASSAY

All the extracts were screened for in vitro anti-microbial activity by disc diffusion method. The sterilized Mueller hinton agar media and sabourd dextrose agar media were heated on the water bath to melt the media, when the media was lukewarm the organism were inoculated separately and 15ml was poured aseptically in to sterile Petri dishes and allowed to solidify. The extracts were dissolved and diluted with dimethylsulfoxide. Then sterilized discs were prepared at 800µg/disc for each leaf extracts and 10µg/disc for standard antibiotics (ciprofloxacin and clotrimazole) separately. The zone of inhibition was compared with standard drugs after 24hrs of incubation at 37^0c for bacteria's and 48hrs of incubation at 30^0 c for fungi.

BROTH MICRO-DILUTION ASSAY

The leaf extracts are diluted two fold concentration for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) by micro broth dilution method in Mueller Hinton or Sabouraud broth. The leaf extracts and standard antibiotics (ciprofloxacin and clotrimazole) were dissolved in 1% DMSO aqueous solution at concentration of 10 mg/ml. These diluted extracts solutions (with Solvent DMSO) were used in the

determination of the antimicrobial activity against the reference strains. The extracts were diluted two fold concentration from 200 to >2400 µg/ml and the starting inoculums of 2.0 $\times 10^7$ CFU/ ml were used. The tubes were incubated at 37°C for bacteria and 30°C for fungi. For the evaluation of MBC/MFC, a portion of liquid (5 µl) from each extracts that showed no change in color was plated on MHA and incubated at 37 °C for 24 h. The lowest concentration of extracts that yielded no growth after this sub-culturing was taken as the MBC/MFC.

Table 1. Phytochemical Screening of *Tribulus subramanyamii*

Name of extract	Carbohydrates	proteins	tannins	Alkaloids	sterol	Flavonoids	glycosides	Saponins
Ether	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
chloroform	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve
Alcohol	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve

Table 2. Antimicrobial activity of the leaf extracts and reference antibiotic determined by the agar disc diffusion assay

Leaf extracts	Zone of inhibition diameters in mm			
	Bacillus cereus MTCC-1306	Escherichia coli MTCC-118	Aspergillums Niger MTCC-1785	Candida albicans MTCC-183
Ether	5.2 ± 0.2	3.4±0.2	2.1±0.2	2.4±0.1
Chloroform	8.5 ± 0.1	5.0±0.1	2.0±0.1	2.5±0.2
Ethanol	10.5±0.1	6.2±0.1	2.0±0.1	2.0±0.1
Ciprofloxacin	14.5±0.2	14.5±0.2	-	-
Clofrimazole	-	-	15.2±0.1	15.5±0.2

Values are given as mean ± SD (n=2), (-); not tested Anti-microbial activity leaf extract 800µg/disc and standard antibiotics 10 µg/disc.

Table 3. Minimum inhibition concentration (µg) of leaf extracts and reference antibiotics determined by the broth micro dilution method

Leaf extracts	Minimum inhibition concentration (MIC/in µg)ml			
	Bacillus cereus MTCC-1306	Escherichia coli MTCC-118	Aspergillums Niger MTCC-1785	Candida albicans MTCC - 183
Ether	500	>600	>1200	>1200
Chloroform	400	>600	>1200	>1200
Ethanol	300	600	>1000	>1000
Ciprofloxacin	0.8	0.8	-	-
Clofrimazole	-	-	0.8	0.8

(-); not tested

Table 4. Minimum bactericidal or fungicidal concentration (MBC/MFC) (µg) of the leaf extracts and reference antibiotics determined by the broth micro dilution method

Leaf extracts	Bacillus cereus MTCC-1306	Escherichia coli MTCC-118	Aspergillums Niger MTCC-1785	Candida albicans MTCC-183
Ether	1000	>1200	2400	2400
Chloroform	800	>1200	2400	2400
Ethanol	600	1200	2000	2000
Ciprofloxacin	1.6	1.6	-	-
Clofrimazole	-	-	1.6	1.6

(-); not tested

RESULT AND DISCUSSION

Our study is based on the both extraction and phytochemical screening of the *Tribulus subramanyamii* Linn. The investigation shows the presences of alkaloids, Steroids and saponins in the chloroform leaf extract. The Ethanolic leaf extracts showed the presence of Carbohydrates, Steroids, Glycosides and Saponins. The ether extract indicates the presence of steroids and saponins (Table1). Then our work extended to study invitro anti-microbial activity of the ether, chloroform, and ethanolic leaf extracts of *Tribulus subramanyamii* against gram positive bacteria; gram negative and fungal species by the disc diffusion method. The results of anti-microbial activity of leaf extracts and reference drugs by agar disc diffusion assay are summarized in Table-2. It has been investigated all the leaf extracts has moderates to good activity against bacillus cereus and moderate activity against E coli when compared with the standard drug ciprofloxacin. All the leaf extracts were source less effective zone of inhibition against aspergillus niger and Candida alibicans. The results of MIC of leaf extracts and reference drugs are recorded in Table-3. It shows the MIC values range in form 300-500µg/disc, 600->600µg/disc for E.coli, 1000->1200 µg /disc for aspergillus Niger, >1000->1200µg/disc for Candida albicans. The results of minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) determined for various leaf extracts, are displaced in Table-4. All the extracts displayed the MBC values between 600-1000µg/disc for bacillus cereus, 1200->1200µg/disc for e coli .All the extracts shows the MFC range in between 2000-2400µg/disc for aspergillus niger and Candida albicans respectively.

CONCLUSION

Phytochemical investigation of leaf extracts of *Tribulus subramanyamii* Linn shows the presence of carbohydrates, alkaloid, steroids, and saponins. All the extracts are shows appreciable activity against gram positive bacteria, bacillus cereus. All the extracts were displayed moderate activity against gram negative bacteria, e-coli. It was observed that as the extracts not so effective against fungal species, it is possible say the leaf extracts had better activity against bacteria than fungi.

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