

Detection of Phytochemical and Pharmacological Properties of Crude Extracts of *Tribulus terrestris* Collected from Tribal Regions of Baglan (M.S.), India

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ABSTRACT

Nature is rich source of medicinal plants and herbal remedies but due to inadequacy of information about their chemical and therapeutic properties some of these sources are still remained unrevealed. Herbal drugs are the potential source of therapeutic aid for the treatment and prevention of various infections and diseases. Naturally occurring shrub *Tribulus terrestris* was collected from tribal area of Baglan Region (Nashik District). The extracts of plant parts such as leaves and fruits were prepared in water, acetone and chloroform. The detection of important phytochemicals of plant *Tribulus terrestris* was attempted. Each crude extract of leaves and fruit of plant *Tribulus terrestris* was comparatively analyzed for presence of phytoconstituents. Phytochemical analysis of crude extracts of *Tribulus terrestris* showed presence of carbohydrates, amino acids, proteins, glycosides, tannins, terpenoids, phenols, alkaloids and saponins.

Keywords: *Tribullus terrestris*, Phytochemicals, Baglan, Bokharu.

INTRODUCTION

Soil is a dynamic body on earth which provides nutrients for growth of the plants. Plants are rich source of food and other valuable ingredients for humans. Medicinal plants have been the mainstay of traditional herbal medicine amongst rural inhabitants worldwide since ancient time¹. Humans used plants for a variety of purposes. Most of medicines are prepared from natural ingredients, specifically from plants sources. India is the richest country in its plant biodiversity. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicines²⁻⁴. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 percent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care¹. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances³. Due to indiscriminate use of synthetic antimicrobials, the development of resistant and multi resistant pathogens have been observed⁵⁻⁶. Natural therapeutics which are derives from plants are most significant because of lesser side effects, effective, cost effective and ecofriendly as compared to chemically synthesized medicines. The concentration of phytochemicals is different in different plants and also in different in different parts of the same plant⁷. Phytochemicals are regarded as secondary metabolites because the plants that manufacture them may have little need for them⁸. The therapeutic efficacy of plants depends

on their production of secondary compounds such as alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, tannins, etc⁷. Plant *Tribulus terrestris* belongs to Family- Zygophyllaceae, common name- Bokharu (also known as puncture vine, caltrop, and yellow vine) is common shrub found in India. It is an annual plant found in warm and dry tropics of Asia, Africa Europe, America and Australia⁹. The pharmacological analysis of *Tribulus terrestris* is mentioned in few reports from different regions of globe. The geographical distribution and atmospheric conditions may play an important role in growth and physiological activities of plants. In present study the detection of phytochemical constituents of leaves and fruits extracts of *Tribulus terrestris* collected from tribal area of Baglan (M.S.) India was attempted.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of the *Tribulus terrestris* were collected from tribal region of Baglan District Nashik, M.S., India and brought to laboratory. The plant was authenticated by Dr. Balasaheb R. Pawar, Taxonomist and Head, Department of Botany of Institute. Plant parts leaves and fruits were cleaned by washing with distilled water to remove dust, and other contaminants. The leaves and fruits were shade dried into laboratory at room temperature for 10 days. The dried leaves and fruits were grounded to make fine powder. Powdered samples were kept in air tight containers.

Extraction of plant material

The plant material extracts were prepared according to Sasikala *et al.*, 2014. with some modifications.

Aqueous extract

Fine powder of leaves and fruits was dissolved in sterile distilled water (10% w/v) separately in Erlenmeyer flask to make aqueous extract. The flasks were placed on orbital shaker for 48 hours for extraction process. The extract was then evaporated in a rotary evaporator at 60°C¹¹. The final dried samples were stored in labeled sterile bottles and kept at 4°C.

Methanol extract

Each dried plant sample was ground and extracted with methanol. 20 gm of powder was soaked into 200 ml of methanol. The extraction process was carried out for 48 hours at room temperature on orbital shaker. The ethanol extract was dried under a reduced pressure at 40°C. The dried extracts were stored in sterile bottles until further use.

Acetone extract

Powdered sample (20 g) from plant samples were extracted with acetone by continuous agitation for 48 hours. The solvent was removed using a rotary vacuum evaporator at 40°C to give a concentrated extracts, which were then stored at 4°C till use.

Preliminary Phytochemical Analysis of crude extracts

The crude extracts of *Tribulus terrestris* were subjected to preliminary phytochemical screening for the detection of major chemical groups. The different qualitative chemical tests were carried out on the extracts using standard procedures to identify the constituents^{12-13,4,14-18}. The phytochemical analysis of all crude extracts of *Tribulus terrestris* were conducted for detection of carbohydrates, amino acids, proteins, glycosides, tannins, terpenoids, phenols, alkaloids and saponins.

Detection of Carbohydrates

0.5 mg extracts were dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Iodine Test

To 5 ml of the aqueous extract was added with 2 ml of iodine solution. The formation of purple color indicates the presence of carbohydrates.

Benedict's test

To 1ml of the filtrate 5ml Benedict's reagent was added and boiled for 5 minutes. Bluish green color was developed which indicates the presence of carbohydrates.

Detection of Protein and Amino acids

Biuret test

To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of one percent copper sulphate solution was added. The appearance of violet color indicated the presence of protein.

Ninhydrin test

About 0.5 mg of extract was taken and two drops of freshly prepared Ninhydrin reagent was added. The pinkish purple color indicated the presence of peptides or amino acids.

Test for Glycosides

About 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following test

Borntrager's test

To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink color formation indicated presence of glycosides.

Detection of Tannins

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of FeCl₃ solution were added. The formation of a green precipitate was an indication for the presence of tannins.

Detection of Terpenoids

Salkowski's test

About 5 mg of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated H₂SO₄ was carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicated the presence of terpenoids.

Detection of Phenols

Ferric chloride test

10 mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenol.

Lead acetate test

10 mg extracts was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of phenol.

Detection of Saponins

About 0.5 mg of the extract was shaken with 5 ml of distilled water. Formation of stable foam for more than 5 min. showed the presence of saponins.

Detection of Alkaloids

About 2 ml of extracts were taken into a test tube and 1 ml diluted HCl was added. The mixture was heated gently for 20 min. and allowed to cool and filtered. The filtrate was used for following test.

Wagner test

Filtrate was treated with Wagner's reagent; formation of brown reddish precipitate showed presence of alkaloids.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of crude leaves extracts

The therapeutic significance of plants depends on presence of phytochemicals. The secondary metabolites of plants have been proved their therapeutic values which plays wide range of activities in herbal remedies. *Tribulus terrestris* is used for medicinal purposes since old time. Plant *Tribulus terrestris* was used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, and urinary anti-infective¹⁹⁻²¹. The analysis of phytochemicals in crude leaves and fruits extracts of *Tribulus terrestris* showed presence of carbohydrates, amino acids, proteins, glycosides, tannins, terpenoids, phenols and saponins. The phytochemical characteristics of *Tribulus terrestris* leaves and fruits extracts are summarized in Table 1. The present investigation revealed the presence of important secondary compounds in all three extracts. All extracts of leaves and fruits of plant *Tribulus terrestris* showed presence of carbohydrates, proteins and saponins. Saponins of plants have often considered as antibacterial, anti-inflammatory

Table 1: Phytochemical Analysis of Crude Extracts of Leaves and Fruits of *Tribulus terrestris*.

Phytochemicals	Leaves Extracts			Fruit Extracts		
	Aqueous	Methanol	Acetone	Aqueous	Methanol	Acetone
Carbohydrates	+	+	+	+	+	+
Amino acids and peptides	+	+	+	+	+	+
Glycosides	-	+	+	-	+	+
Tannins	+	+	+	+	+	+
Terpenoids	-	+	-	-	+	-
Phenols	-	+	+	-	+	+
Saponins	+	+	+	+	+	+
Alkaloids	-	+	+	-	+	+

(Present: +, Absent: -)

and antitumor activities²²⁻²³. Antibacterial activity of saponins may be due to membranolytic property²⁴⁻²⁵. Flavonoids, alkaloids and terpenoids are considered to be antimicrobial and antidiarrheal¹⁶. Flavonoids have been found in vitro to be effective antimicrobial substances against different microorganisms. The antibacterial activity of flavonoids may be due to their ability to form complexes with extracellular and soluble proteins and also with bacterial cell walls²⁶. The presence of tannin was detected in all extracts of leaves and fruits of *Tribulus terrestris*. Tannins (commonly referred as tannic acid) are water soluble polyphenols present in many plants has antimicrobial effect. Tannins were detected to cause precipitation of microbial proteins²⁷. Tannins are reported to have various physiological effects like anti-irritant, antisecretolytic, antimicrobial and antiparasitic effects²⁸. In methanol and acetone extracts of plant *Tribulus terrestris* were found presence of alkaloids. Heterocyclic nitrogen compounds are called alkaloids. It is reported that alkaloids have microbiocidal effects^{29-30, 24}.

CONCLUSION

On the basis of results obtained in present study, it could be said that *Tribulus terrestris* plant extracts (aqueous, methanol and acetone) contains chemical constituents of pharmacological significance. Further advanced study such as quantitative determination, purification and characterization of phytoconstituents of *Tribulus terrestris* may help to formulate the herbal preparations of medicinal use.

REFERENCES

- Doughari JH: Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents, phytochemicals - A global perspective of their role in nutrition and health. Dr Venketeshwer Rao (Ed.). 2012. InTech ISBN: 978-953-51-0296-0.
- Rajshakaran PE: Herbal medicine in: World of Science. Employment News 2002; 3:21-27 November.
- Veerachari U, and Bopaiiah AK: Preliminary phytochemical evaluation of the leaf extract of five *Cassia Species*. Journal of Chemical and Pharmaceutical Research 2011; 3(5):574-583.
- Udayaprakash, NK, Bhuvanewari S, Preethy S, Rajalakshmi N, Saranya M, A Jasmine R and Arokiyaraj S: Studies on antimicrobial, antioxidant, larvicidal, pesticidal activity and phytochemistry of leaves of *Alangium salvifolium* (L.F.) wang. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5: 86-89.
- Christudas S, Kulathivel TM, and Agastian P: Phytochemical and antibacterial studies of leaves of *Tridax procumbens* L. Asian Pacific Journal of Tropical Biomedicine 2012; S159-S161.
- Karaman L, Sahin F, Gulluce M, Ogutcu H, Sngul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. Journal of Ethnopharmacology 2003; 85: 231-235.
- Jalpa R, Moteriya P and Chanda S: Phytochemical screening and reported biological activities of some medicinal plants of Gujarat region. Journal of Pharmacognosy and Phytochemistry 2015; 4(2): 192-198.
- Nandagoapalan V, Doss A and Marimuthu C: Phytochemical analysis of some traditional medicinal plants. Bioscience Discovery; 2016 7(1): 17-20.
- Kumar A: comparative and quantitative determination of quercetin and other flavonoids in north Indian populations of *Tribulus terrestris* Linn, by hplc. International Journal of Pharma Bio Sciences 2012; 3(4): 69 – 79.
- Sasikala S, Kannan E and Brindha D: Qualitative characterization of solvent and cooked extracts of *Tribulus terrestris* L fruit. European Journal of Medicinal Plants 2014; 4(8): 907-919.
- Kandil O, Radwan NM, Hassan AB, Amer AMM, El-Banna HA and Amer WMM, Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. Journal of Ethnopharmacology 1994; 44(1):19-24.
- Trease GE and Evans WC: Pharmacognosy, Bailliere Tindall, London. Eleventh Edition 1989.
- Harborne JB: Phytochemical methods, Chapman and Hall Ltd., London. 1973.
- Sahira BK and Cathrine L: General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science 2015; 2(4): 25-32.
- Sengottuvel, Santhi R and Kalaiarasi KS: Phytochemical analysis of methanolic extract of *Coccinia grandis* L. International Journal of Scientific Research 2016; 5(9):263-265.

16. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H: Phytochemical screening and extraction: A review. *International Journal of Pharmaceutical Sciences* 2011; 1(1):98-106.
17. Njoku OV and Obi C: Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Applied Chemistry* 2009; 3(11): 228-233.
18. Sawant RS and Godghate AG: Preliminary phytochemical analysis of leaves of *Tridax procumbens* Linn. *International Journal of Science, Environment and Technology* 2013; 2(3): 388–394.
19. Saied K and Fereshteh J: Evaluation of antibacterial activity of *Tribulus terrestris* L. growing in Iran. *Iranian Journal of Pharmacology & Therapeutic* 2003; 2:22-24.
20. Ody P. *The Complete Guide Medicinal Herbal*. London: Dorling Kindersley 2000.
21. Deepak, M, Dipankar G, Prasanth D, Asha MK, Amit A and Venkatraman BV: Tribulosin and β -sitosterol-Dglucoside, the anthelmintic principles of *Tribulus terrestris*. *Phytomedicine* 2002; 9: 753-756.
22. Lakshmi KS, Sangeetha D, Sivamani D, Tamilarasan M, Rajesh TP and Anandraj B: In Vitro antibacterial, antioxidant, haemolytic, thrombolytic activities and phytochemical analysis of *Simarouba glauca* leaves extracts. *International Journal of Pharmaceutical Sciences Review* 2014; 5(2): 432-437.
23. Sparg SG, Light ME and van Staden J: Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology* 2004; 94: 219-243.
24. Al-Bayati FA and Al-Mola HF: Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *Journal of Zhejiang University Science B* 2008; 9(2):154-159.
25. Killeen G, Madigan C, Connolly C, Walsh G, Clark C, Hynes M, Timmins B, James P, Headon D and Power R: Antimicrobial saponins of *Yucca schidigera* and the implications of their in vitro properties for their in vivo impact. *Journal of Agriculture and Food Chemistry* 1998; 46(8): 3178-3186.
26. Lubna F MS (U), Arshiya S MD (U), Saad A MD(U) and Shabiya S MD(U): Pharmacological activities of *Tribulus terrestris* Linn: A systemic review. *World Journal of Pharmacy and Pharmaceutical Sciences* 2015; 4(2): 136-150.
27. Mir AS, Dar MA, Mir S, Ahmad SM, Chitale G : Analysis of phytochemistry and antimicrobial activity of *Tridax procumbens* Linn. *Chemical Sciences Journal* 2016; 7: 132. doi:10.4172/2150-3494.1000132.
28. Dhanabalan R, Doss A, Jagadeeswari M, Ballachander S, Kezia E, Parivuguna V., Reena Josephine CM, Vaidheki R and Kalamani K: In vitro phytochemical screening and antibacterial activity of aqueous and methanolic extract of *Tridax procumbens* against bovine mastitis isolated *Staphylococcus aureus*. *Ethanobotanical Leaflets* 2008 12: 1090-1095.
29. Cowan MM: Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 1999; 12(4): 564–582.
30. Omulokoli E, Khan B, Chhabra S: Antiplasmodial activity of four Kenyan medicinal plants. *J. Ethnopharmacology* 1997; 56(2): 133-137.