



Research Article

PHYTOCHEMICAL SCREENING OF LEAVES, STEM AND FRUIT PARTS OF *TRIBULUS TERRESTRIS*

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Article Received on: 28/01/21 Approved for publication: 27/02/21

DOI: 10.7897/2230-8407.1202121

ABSTRACT

The comparative phytochemical analysis of leaves, stem and fruit powder of *Tribulus terrestris* was carried out by using preliminary phytochemical screening. To identify the phytochemical constituents, present in water and ethanol extracts of *Tribulus terrestris* leaves, stem and fruit, a preliminary analysis was done by using different testing methods of Frothing test, Mayer's test, Hager's test, Foam formation test, Lead acetate test, Molisch's test and Ferric Chloride test. The phytochemical analysis study showed the presence of 3.903%, 1.725% and 2.259% terpenoids & phenolic in leaves, stem and fruit parts of *Tribulus terrestris* respectively. The study showed the presence of 16.574%, 15.943% and 10.168% alkaloids and 0.240%, 0.160% and 0.230% Q. alkaloid & N-oxides in leaves stem and fruit extracts respectively. 2.888%, 0.825% and 2.679% fats and waxes and 76.395%, 81.347% and 84.672% fibers were observed in leaves, stem and fruit extracts of this plant respectively

Keywords: *Tribulus terrestris*, quaternary alkaloids and n-oxides, terpenoids and phenolic, phytochemicals.

INTRODUCTION

Medicinal plants are the important source of pharmaceuticals and healthcare products. Ayurveda, Unani, Homeopathy and Siddha are the traditional system of medicines. It is mainly depending on the natural products.¹ Phytochemicals are important sources of food and medicine. It plays very important role in prevention of illness and to maintain human health.² Medicinal plants products are usually made by plant parts like leaves flowers, tubers, bulbs and roots from different plant species in specific proportions based on the desired function of the concoction³. They are used as home remedies to treat specific conditions as well as complex preparations to treat life threatening diseases.⁴ In twentieth century, antibiotics which have been used against bacterial infection are very significant things, but these synthetic drugs are used to treat only one third of the known infectious diseases.⁵ Recently, antibiotic resistance has increased on large scale and it is going to be a big therapeutic problem. The problem can be solved with the help of antibiotic resistance inhibition from plants.^{6, 7} Trees produces different compounds to protect themselves from various pathogens. Plant extracts are expected to be active against drug resistance pathogens.⁸ For hundreds of years, different plants have been used to treat various human illnesses in many parts of the world traditionally. So, phytomedicines and biologically active compounds derived from plants which are used in herbal medicines have been focused by researchers.⁹⁻¹⁰ *Tribulus terrestris* is a flowering plant of the *Zygophyllaceae* family. The plant observes in warm temperate and tropical regions of Southern Europe, southern Asia, Africa, and Australia. It is useful in the traditional medicine of many countries for treatment of cardiac diseases, acute conjunctivitis, headache, edema, skin itch, vertigo, abdominal distention. It is also used in the folk medicine of India in Ayurveda for its diuretic, anti-urolithiatic, and aphrodisiac properties. Local Bedouin use the plant in the treatment of impotency, urinary disorders and liver diseases. The seeds are used to treat kidney

stone, hemorrhages and gout. The fruit is considered to be tonic, aphrodisiac, diuretic and hepatoprotective. The other activities reported are in infertility therapy, anti-inflammatory, antioxidant, cardio protective, antitumor, antispasmodic and anti-hyperglycemic.

MATERIALS AND METHODS

Preliminary Phytochemical Analysis:

To identify the phytochemical constituents, present in water and ethanol extracts of *Tribulus terrestris* leaves, stem and fruits a preliminary analysis was done by using different testing methods of Frothing test, Mayer's test, Hager's test, Foam formation test, Lead acetate test, Molisch's test and Ferric Chloride test.

Saponins

Frothing test: 2 cm³ of the extract was taken in a test tube. The test tube was vigorously shaken for two minutes. Frothing observed in the water extract of leaves, stem and fruits indicated the presence of saponins.¹²

Tannins

1 ml of freshly prepared 10% KOH was added to 1 ml of the water and ethanol extract of leaves, stem and fruits. A dirty white precipitate noticed in all the extracts except water extract of fruit indicated the presence of tannins.

2 drops of 5% FeCl₃ were added to 1 ml of water and ethanol extracts. The formation of very dark greenish colored precipitate indicated the presence of tannins in water and ethanol extracts of leaves, stem and fruits.¹²

Alkaloids

2 drops of Mayer's reagent were added to 1ml of water and ethanol extract of each leaves, stem and fruits. A creamy

precipitate which was noticed in all the extract except water extract of leaves and ethanol extract of stem. It showed the presence of alkaloids.

2 drops of Hager's reagent were added to 1ml of water and ethanol extract of each part of plant in separate test tubes. A reddish-brown precipitate observed, it confirmed the presence of alkaloids.¹³

Flavonoids

1 ml of 10 % NaOH was added to 3 ml of water and ethanol extract. A yellow coloration proved the presence of flavonoids in *Tribulus terrestris* leaves, stem and fruits.

2 ml of aqueous and ethanol extracts were added into a test tubes and a few drops of concentrated ammonia were added, the formation of a yellow coloration showed the presence of flavonoids in leaves, stem and fruits.¹²

Carbohydrates

To ascertain the presence of carbohydrates, few drops of molisch's reagent were added to 2 ml of water and ethanol extract. 1 ml of concentrated sulphuric acid was allowed to run down the inclined test tube to form a lower layer. The interface was observed for a purple colour in each extract instead of water extract of leaves and ethanol extract of fruits. It confirmed the presence of carbohydrates in each part of *Tribulus terrestris*.¹²

Protein

2 ml of aqueous and ethanol extract was added into a test tube and a few drops of concentrated HNO₃ were added, the absence of orange colour except water extract of leaves showed the absence of proteins in *Tribulus terrestris*.¹⁴

Phenolic compounds

2 ml of water and ethanol extract of leaves, stem and fruits were added into a test tube and a few drops of alcohol were added, then 2 drops of 5% FeCl₃ were added in it. The formation of Greenish yellow colour was observed only in leaves extracts indicates the presence of phenolic compounds only in leaves part of *Tribulus terrestris*.¹⁴

Quinine

2 ml of aqueous and ethanol extract of each part was added into a test tubes and a few drops of HCl were added, the absence of green colour showed the absence of quinine in leaves, stem and fruits of *Tribulus terrestris*.¹⁴

Extraction

Extraction was done by using following procedure.^{15,24}

Accurately weighed 20 grams of the *Tribulus terrestris* leaves powder was continuously extracted in Soxhlet apparatus for 30 hrs. The 500 ml mixture of methanol and water in volume ratio 4:1 was used as extractant. The extract was cooled and filtered by using Whatman filter paper no.41 into a dry and preweighed beaker. From the residue fats and waxes were separated first by using ethyl acetate solvent. The further separation was made by using separating funnel. The filtrate was acidified with the help of 2M H₂SO₄. The acidified filtrate was again extracted by using 150 CC (3 x 50 CC) chloroform in a separating funnel. The chloroform layer obtained was the moderately polar extract. It consists of terpenoids. The aqueous layer obtained was basified to pH-10 with 2M NaOH. It was again extracted with 120 CC (2 x 60 CC) chloroform: methanol in volume ratio 3:1 followed by extraction with 80 CC (2 x 40 CC) chloroform in a separating funnel. The aqueous basic layer was collected in a dry preweighed beaker. The methanol layer contains quaternary alkaloids and N-oxides and the chloroform extract was the basic extract. It consists of alkaloids. The same procedure was repeated for both stem and fruit powders.

RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical Analysis of extracts of *Tribulus terrestris*

Phytochemical constituents	Leaves		Stem		Fruit	
	Water extract	Ethanol extract	Water extract	Ethanol extract	Water extract	Ethanol extract
Saponins	++	-	++	-	++	-
Tannins	++	+	+	+	-	+
Alkaloids	-	+	+	-	+	+
Flavonoids	++	+	++	+	+	++
Carbohydrates	-	+	+	+	+	-
proteins	+	-	-	-	-	-
Phenolic comp.	++	+	-	-	-	-
quinine	-	-	-	-	-	-

++ = High concentration; + = Low concentration; - = Absent

Table 2: % yield of phytochemicals of *Tribulus terrestris* after extraction

Sr. No.	Parameters	Phytochemical Analysis of Leaves		Phytochemical Analysis of Stem		Phytochemical Analysis of Fruit	
		Weight in gm	Percentage	Weight in gm	Percentage	Weight in gm	Percentage
1	Fats and waxes	0.578	2.888%	0.165	0.825 %	0.536	2.679%
2	Terpenoids & Phenolics	0.781	3.903%	0.345	1.725%	0.452	2.259%
3	Q. Alkaloid & N - Oxides	0.048	0.240%	0.032	0.160 %	0.046	0.230%
4	Alkaloids	3.317	16.574%	3.189	15.943%	2.034	10.168%
5	Fibers	15.289	76.395%	16.272	81.347 %	16.937	84.672%
	Total	20.013	100.00%	20.003	100.002%	20.003	100.008%

Each observation is the mean of three readings.

Phytochemical analysis of *Tribulus terrestris* were carried out in this research. The preliminary phytochemical screening (Table. 1) revealed the presence of saponins, tannins, alkaloids, flavonoids, carbohydrates in leaves, stem and fruits parts in more or less proportion. The proteins and quinine are observed absent in each part except water extract of leaves. Phenolic compounds were observed present only in leaves extracts. Saponins were not detected in the ethanol extract. These constituents present in the leaves, stem and fruit extracts have good therapeutic values. Saponins and terpenes have antimicrobial and curative properties against various pathogens.¹⁶ Flavonoids have antifungal as well as antibacterial activity. They also possess anti-inflammatory property.¹⁷ Tannins and saponins are known to have antimicrobial properties.¹⁸ Secondary metabolites of the plants like alkaloids, terpenoids and glycosides play the role of protective agents against different pathogens like insects, fungi or bacteria. They also function as growth regulatory molecules such as hormone like substances. Consequently, they are used as potential anticancer drugs by direct cytotoxic activity against cancer cells or by reducing the tumor development process.¹⁹

The extraction % yield (Table. 2) showed the presence of 3.903%, 1.725% and 2.259% terpenoids & phenolic in leaves, stem and fruit parts of *Tribulus terrestris* respectively. In Pharmaceutical and food industries, terpenes are used as medicines and flavor enhancer because of their potentials and effectiveness. As antibiotic resistant bacteria are being increased globally, terpenes are important.²⁰ The group of terpenoids exhibits various pharmacological activities like anti-viral, anti-malarial, anti-inflammatory and anti-cancer activities. It also inhibits cholesterol synthesis.²¹

The study showed the presence of 16.574%, 15.943% and 10.168% alkaloids and 0.240%, 0.160% and 0.230% Q. alkaloid & N-oxides in leaves, stem and fruit extracts respectively (Table.2). Alkaloids govern plant growth. Different alkaloids show different medicinal properties such as Caffeine is stimulant; Codeine is cough medicine and analgesic; Quinidine is antiarrhythmic; Quinine is antipyretics and antimalarial; Reserpine is antihypertensive etc.²² 2.888%, 0.825% and 2.679% fats and waxes and 76.395%, 81.347% and 84.672% fibers were observed in leaves, stem and fruit extracts of this plant respectively (Table.2). Lipids are useful in Nutrition and Dietary, Food Science, cosmetics, pharmaceuticals, paints and varnishes, detergents in human society.²³

CONCLUSION

The results of the present study showed that the crude ethanolic and aqueous extracts of *Tribulus terrestris* leaves, stem and fruits contain saponins, tannins, alkaloids, flavonoids and carbohydrates. Phenolic compounds were observed present only in leaves extracts. The percentage of Fats and waxes, Terpenoids and Phenolics, Q. Alkaloid & N – Oxides was observed in good quantity. *Tribulus terrestris* is full of important phytochemicals and nutritional constituents in noticeable amounts. The presence of phytochemicals can make this plant as potential drug

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Cite this article as:

Nirmala R. Kakade et al. Phytochemical screening of leaves, stem and fruit parts of *Tribulus terrestris*. Int. Res. J. Pharm. 2021;12(2):19-22.

<http://dx.doi.org/10.7897/2230-8407.1202121>

Source of support: Nil, Conflict of interest: None Declared

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