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Original Research Article

STUDY ON THE PHYTOCHEMICAL ANALYSIS AND DETERMINATION OF SECONDARY METABOLITES ON SEED OF *TRIBULUS TERRISTRIS*

Paridhi Nyati[1]*, Shilpa Bhargava[2]

[1,2]Department of Biotechnology, Biyani Girls College, Jaipur-302039, Rajasthan, India

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Corresponding author: Paridhi Nyati

Conflict of interest: Nil

Abstract

The present research work the systematic valuation of *Tribulus terrestris* for its medical efficacy. The aim of this study is to evaluate secondary metabolites phytoconstituents of seed powder of *Tribulus terrestris* qualitatively as well as quantitatively. Phytochemical screening of seed extract of *Tribulus terrestris* carried out with aqueous solvent. Phytochemical screening revealed the presence of phenol, Saponin, glycosides, carbohydrate, Flavonoid, alkaloids, protein and tannin. The quantitative studies revealed that seed of *Tribulus terrestris* possessed Alkaloids, Tannin, Saponin, cardiac glycoside and Flavonoid value was found to be 39.6%, 30.4%, 0.19%, 25.9%, and 32.1% respectively. The high level of primary metabolites in the sample reveals its nutritive value. These secondary metabolites could be responsible for the observed medicinal properties of *Tribulus terrestris* by traditional herbalists.

Keywords: *Tribulus terristris*, primary metabolites, secondary metabolites, medicinal properties

INTRODUCTION

For humans, plants are a significant source of food and other vital ingredients [1]. India is a home to a diverse range of plants and flora. A botanical survey of India had found about 46000 plant species. A whopping 7000 plants possessed therapeutic qualities [2]. The World Health Organization (WHO) defines a medicinal plant as any plant that contains compounds that can be utilised therapeutically or that serve as a precursor to new semi-synthetic chemo-pharmaceuticals. The use of medicinal plants significantly influenced both individual and communal health [3]. Since ancient times, medicinal plants and herbs have had a substantial impact on global health [2]. The modern health care delivery system can obtain adequate drugs from plants. Plants have two main purposes: first, they are utilized to make medicines, and second, they serve as a sort of organic model for the development of new therapeutics [4]. Any herbal preparation made from plant materials through extraction, fractionation, purification, concentration, or other physical or biological processes may be produced for immediate use or utilized as the foundation for subsequent herbal products. Medicinal plants have been utilized to treat human diseases for thousands of years because they contain a wide variety of chemical substances that can have a specific physiological effect on people [5]. Due to the availability of physiologically organic substances, more

than 35,000 plant species are consumed for medical purposes across all of the human communities. In all plant cells, bioactive substances often accumulate as secondary metabolites, however the composition varies depending on the plant section. Phytochemicals are still employed in both traditional and advanced medicine and are crucial in the treatment among several different diseases and disorders. For the purpose of plant defense and adaptability to environmental stress, they are produced via certain metabolic pathways [6]. Phytochemicals are plant compounds that are not edible yet have the ability to fend off disease [7]. A medicinal plant seems to have a variety of phytochemicals or secondary metabolites that either work alone, together, or in combination to promote health [6]. The main bioactive components of plants are phenolic chemicals, alkaloids, tannins, and flavonoids [3]. A tool for evaluating the quality of the plant's components individually or as a whole is phytochemical screening. Various qualitative tests using precipitation and colour reaction exhibit the presence or absence of bioactive compounds [2].

Tribulus terrestris L., commonly known as puncture vine, caltrop, yellow vine, goat head and devil's horn. It belongs to family Zygophyllaceae and is widely distributed in both tropical and mild temperate regions [8]. It is a small, prostrate, 10–60 cm high, hirsute or silky hairy shrub. The leaves are opposite, often unequal, par pinnate, pinnate from 5 to 8 pairs and elliptical Oran oblong lanceolate. The fruits from the five mericarps are x-shaped, 3–6 mm long, and arranged radially and have a diameter of 7–12 mm and a hard texture. Root is slender, fibrous, and cylindrical and frequently branched and also bears a number of small rootlets and has light brown colour [9]. There are several seeds in each crocus with transverse partitions between them. The seeds are oily in nature. *Tribulus terrestris* had been used in folk medicines as tonic, aphrodisiac, palliative, astringent, stomachic, antihypertensive, diuretic, lithotriptic, and urinary disinfectant [10]. The extract of drug is also used in the treatment of nephritis and kidney stones for painful micturation and for treatment of gout as well [11]. The seeds are useful in epistaxis, haemorrhages and ulcerative stomatitis [6].

MATERIAL AND METHODS

Collection of plant sample

Tribulus terrestris seeds were procured from the University of Rajasthan's botanical garden in Jaipur in decent shape and without disease.

Preparation of Plant extract

The *Tribulus terrestris* seeds were thoroughly washed with clean water, shade dried, and then ground into a fine powder using an electric grinder. The dried powdered sample was then kept at ambient temperature in airtight containers for additional research. In the Soxhlet apparatus, 25 g of the powder have been successively extracted for 24 hours with 250 ml each of distilled water. The solvent was then excluded using a vacuum evaporator, and the crude extracts were then maintained at 4°C in sterile bottles until the analysis was carried out.

Chemicals/Reagents

The chemicals purchased from HiMedia Laboratories Private Limited were all of analytical grade and were used for the experiments (Mumbai, India).

Phytochemical Screening

The plant extract was subjected to a standard phytochemical analysis to check for the presence of alkaloids, steroids, saponins, flavonoids, tannins, tenpins, phenol compounds, and cardiac glycosides.

Test for Alkaloids

The aqueous extract was diluted in acidic solution with 4-5% HCl. Alkaloid detection was done by this solution using various reagents.

Mayer's Test

The Mayer's reagent was combined with 1.0 to 2.0 ml of the test solution. The

presence of alkaloid in the sample was confirmed by the formation of white or buff precipitates.

Wagner's Test

The Wagner's reagent was combined with 1.0 to 2.0 ml of the test solution. The presence of alkaloid in the sample was confirmed by the brown precipitates that formed.

Dragendorff's Test

Dragendorff's reagent was combined with 1.0 to 2.0 ml of the test solution. The presence of alkaloid in the sample was confirmed by the formation of bright orange precipitates.

Hager's Test

Hager's reagent was combined with 1.0 to 2.0 ml of the test solution. The presence of alkaloid in the sample was confirmed by the appearance of yellow precipitates.

Test for Flavonoids

The aqueous extract of the plant was used for the following test

Lead Acetate Test

0.2 gm of plant extract was taken in a test tube and few drops of 10% lead acetate solution were added. Formation of yellow coloured precipitate indicated the presence of flavonoids.

Alkaline reagent Test

The crude extract was mixed with 4.0 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on the addition of dilute acid which indicated the presence of flavonoids.

Test for Carbohydrate

0.2 gm aqueous extract of plant was used to test for the presence of carbohydrate's test.

Molish Test

Few drops of alcoholic solution of α-napthanol was added to 2.0 ml of plant extract the mixture was shaken well and 2 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to rest. Purple or Reddish violet ring appearing at the junction of two liquid indicated the presence of carbohydrate.

Fehling Test

Equal volume of Fehling A and Fehling B was added to the 3.0 ml plant extract and gently boiled in a water bath. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugar.

Barfored's Test

2.0 ml of plant extract was mixed with Barfored reagent and heated on boiling water bath for 2 min. Red precipitate indicated presence of sugar.

Benedict Test

To 1.0 ml of extract, 1.0 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. the appearance of Red/ Yellow/ green/orange-red/ reddish brown precipitate formed which indicated the presence of reducing sugar.

Test for Glycosides

Liebermann's Test

To the extract, 2.0 ml of chloroform and concentrated acetic acid was added in an ice bath. Then 2 drops of concentrated H_2SO_4 was added. Formation of violet to the green colour indicates presence of glycoside.

Keller-Kilani Test

To the extract, 2ml of glacial acetic acid and 2 drop of 2% $FeCl_3$ was added. 2.0 ml of concentrated H_2SO_4 was added along the side of test tube. A brown ring indicates presence of glycoside.

Borntrager's Test

To 2.0 ml of extract, 3.0 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it rose pink color indicated the presence of glycosides.

Salkowski's Test

To 2ml of chloroform and 2.0 ml of H_2SO_4 was added and shake gently. A reddish-brown color indicated the presence of steroidal ring that is glycone portion of the glycoside.

Test for Saponins

Foam Test

To small amount of extract, little quantity of water was added. If foam produced on

shaking persists for 10 minutes, indicates presence of Saponins.

Froth Test

To the 5.0 ml of extract, few drops of sodium bicarbonate solution was added. Mixed vigorously and left undisturbed for 3 minutes. Honey comb like froth is formed.

Test for protein and amino acids

Millon's Test

To 3.0 ml of extract few drops of millon reagent was added. A white/yellow precipitate indicated the presence of proteins.

Ninhydrin Test

Few drops of ninhydrin solution was added to 4.0 ml of aqueous filtrate. A characteristic Purple/blue color indicated the presence of amino acid.

Biuret Test

To 3.0 ml of filtrate, 6 drops of 1% copper sulphate solution was added. A quantity, 3.0 ml of 40% NaOH was also added and the test tube shaken vigorously to mix the contents. A purple/ violet color shows the presence of proteins.

Xanthoprotein Test

To 0.2 ml extract few drops of conc. Nitric acid was added. Formation of yellow color indicates the presence of protein.

Test for Phytosterols

Salkowski's Test

To 2.0 ml extract few drops of conc. Sulphuric acid was added, the mixture was shaken and allowed to stand. Appearance of golden yellow/ yellowish color with green indicates the presence of phytosterol.

Libermann Burchard's Test

2.0 ml filtrates were treated with few drops of acetic anhydride, boiled and cooled and then conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterol.

Test for Phenol and Tannins

Ferric chloride Test

2.0 ml of extract was treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Liebermann's Test

1.0 ml extract was heated with sodium nitrite, H_2SO_4 solution was added and was diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue color indicates the presence of phenols.

Lead acetate Test

1 ml of extract was treated with few drops of lead acetate solution. Formation of cream gelatinous precipitate indicates the presence of tannins.

Quantitative analysis of Secondary Metabolite

Determination of Alkaloids

In 250 ml beaker the 10 gm of plant sample was collected and 200 ml of 10% acetic acid in ethanol was added in the sample.it was kept for 4-5 hours at 28°C. After that it was filtered out with the Whatman filter paper and the filtrate was kept at water bath with ¼ volume of the original volume of sample. After this the droplets of concentrated NH_4OH was added until it was allowed to settle down then the precipitate was washed with the dilute NH_4OH and it was again filtered out by Whatman filter paper. The filtrate was dried at 40°C in hot oven and the remaining extract was calculated for the presence of alkaloid by the formula

%yield= W2-W1/Wt. of sample * 100

W1= Weight of evaporating dish

W2=Weight of dish + Sample

Determination of Flavonoids

15 gm of the plant sample was extracted again and again by 150 ml of 70% aqueous methanol at the room temperature. The solution was filtered with the help of Whatman filter paper. The filtrate was then transferred to the crucible and was evaporated until the dryness over a water bath. The flavonoid content was then calculated using the formula

%yield= W2-W1/Wt. of sample * 100

W1= Weight of evaporating dish

W2=Weight of dish + Sample

Determination of Saponins

150 ml of 10% aqueous ethanol was added to a conical flask along with 10 gm of each

sample. For four hours, the sample was heated in a hot water bath with stirring steadily at 50°C. The filtrate obtained was re extracted with the 200 ml of 20% Ethanol. Then it was reduced to 40 ml at 90°C over the water bath and then the concentrate was transferred to the conical flask and 20 ml of diethyl ether was added in it in a separating funnel. The ether layer was discarded and the aqueous layer was used. The obtained layer was again mixed with the diethyl ether. The 15 ml of n-butanol was added to the solution and was washed twice with the 5% NaCl with the volume measuring of 10 ml. The remaining solution was heated over a water bath. Then it was dried in oven until a constant weight was obtained.

The saponins content was calculated as

%yield= W2-W1/Wt. of sample * 100

W1= Weight of evaporating dish

W2=Weight of dish + Sample

Determination of Tannin

In the flask 500 mg of plant sample was added with the 50 ml of distill water and was shaken for one hour with the help of mechanical shaker. The solution was then filtrated out and 5 ml of filtrate was pipette out in a test tube in which ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M Potassium Ferro cyanide was added. After this its absorbance peak was measured at 520 nm for 10 minutes.

It was calculated as

% yield = Weight of extract / Weight of plant material *100

Determination of Cardiac Glycosides

1.0 gm of plant sample was soaked in the 10 ml of 70% alcohol for 2 hours and then was filtered out. The purification of filtrate was done by lead acetate and Na_2HPO_4. The difference between the intensity of colours of the sample and blank samples (distilled water + Buljet's reagent), gives the absorbance of 595 nm using a suitable spectrophotometer. The cardiac glycosides content was

Calculated as

% yield = Weight of extract / Weight of plant material *100

Result

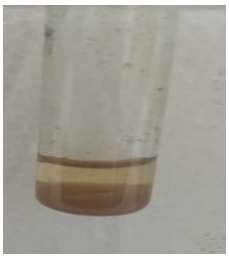
The plants therapeutic significance depends on presence of phytochemicals. The therapeutic properties of plant secondary metabolites, which have a wide range of roles in herbal remedies, have been revealed. Alkaloids, tannins, flavonoids, and a number of other phytochemicals that occur naturally in most plants are known to have medicinal value and can be used to create a defense mechanism against numerous microorganisms. Several environmental factors, such as light intensity, drought stress, temperature, and salinity, have an impact on the presence of secondary metabolite compounds in the plants. The secondary metabolites include both life-threatening poisons and agents with therapeutic value. Many of the isolated secondary metabolites from plants are used in the pharmaceutical industry to produce drugs. In the present study the phytochemical screening of *Tribulus terrestris* seed is done which revealed the presence of various bioactive compounds. Preliminary phytochemical screening shows the presence of Alkaloids, Flavonoids, Carbohydrates, Glycosides, Saponins, Protein & Amino acid, Phytosterol and Phenol & Tannin (Table 1). Different color of the crude extract in phytochemical analysis test was observed (Figure 1). These classes of compounds are known to have curative activity against a variety of pathogens, which may explain their widespread use in the treatment of a wide range of illness. The quantitative determination of Phyto constitutes was done which is summarized in the Table 2.

Table 1: Phytochemical Screening of *Tribulus terrestris* seed

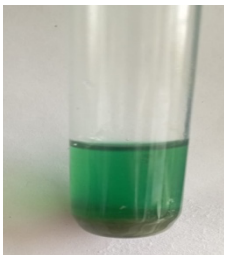
S. No.	Constituents	Test	Result
1.	Alkaloids	Mayer's Test	+
		Wagner Test	+
		Hager Test	+
2.	Flavonoids	Lead Acetate Test	+
		Alkaline Reagent Test	+
		Molish Test	+

3.	Carbohydrates	Fehling Test	+
		Barfored Test	+
		Benedict Test	+
4.	Glycosides	Borntrager Test	-
		Libermann Test	+
		Salkowski Test	+
		Killer Kilani Test	-
5.	Saponins	Forthing Test	+
		Foam test	+
6.	Protein & Amino acid	Millon Test	+
		Ninhydrin Test	-
		Biuret Test	-
7	Phytosterol	Salkowski Test	+
		Libermann Burchard Test	-
8.	Phenol & Tannin	Ferric Chloride Test	-
		Libermann Test	-
		Lead acetate Test	+

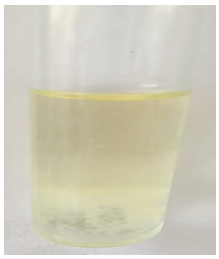
Alkaloid Test



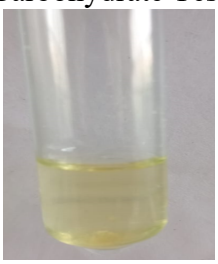
Flavonoids Test



Carbohydrate Test

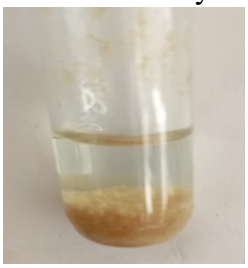


Glycosides Test



Protein Test

Phytosterol Test



Phenol and tannins Test

Figure 1: Phytochemical analysis of *Tribulus terrestris* seed

Table 2: Secondary Metabolites of *Tribulus terrestris* seed

Secondary Metabolites	Mean and Standard deviation (mg/g)	Mean Volume % yield
Alkaloids	1.14±0.01	39.6%
Flavonoids	2.41±0.02	30.4%
Tannin	1.07±0.01	0.19%
Saponin	4.15±0.03	25.9%
Cardiac glycosides	3.15±0.02	32.1%

Discussion

Plants have a nearly limitless ability to manufacture aromatic chemicals. At least 12,000 of them have been isolated, with secondary metabolites making up the majority of them [12]. Because of their capacity to synthesise a variety of secondary metabolites, medicinal plants have been employed for therapeutic purposes since antiquity [13]. The defensive mechanisms of plants against herbivores, insects, and microbiological predators usually consist of these substances [12]. Due to their low cost and accessibility, herbal medications were used around the world.

Plants had formed the foundation of the traditional medical system for thousands of years. The conventional understanding of medicinal plants had been directing the hunt for novel treatments [14]. The results of preliminary phytochemical investigations can be used to determine the plant components that are pharmacologically active. The qualitative study of plants is crucial for locating and isolating the active chemicals that are present in plants. For the pharmacological analysis of newer plant-based medications, the qualitative phytochemical screening of plant extract provides crucial information regarding the chemical composition of plants [15]. Additionally, it aids in the synthesis of novel chemical compounds by researchers [2]. The results of the current study's phytochemical examination showed that alkaloids, flavonoids, tannin,

protein, amino acids, and carbohydrate were present. The existence of saponins, steroids, cardiac glycosides, resins, phenols, terpenoids, glycosides, flavonoids, alkaloids, phenolic compounds, and tannins had been established in earlier studies [13]. One study also noted the presence of glycosides, tannins, terpenoids, phenols, and saponins in addition to carbohydrates, amino acids, and proteins.1One study had also reported the presence of saponins, reducing sugars, triterpenoids, steroids, tannins and alkaloids which was higher in aqueous crude extract of plant as compared to other solvents [16]. Another study had also supported the result of phytochemical analysis of *Tribulus terrestris* which had also confirmed the presence of alkaloid, tannins, glycosides, flavonoids, steroid, phenolic [17]. A plant species fitness for survival is aided by alkaloids. They are utilized as both prescription medications and recreational drugs and frequently have pharmacological effects. Similar to antioxidants and antimicrobials, tannins are well known for their skin-regenerating, anti-inflammatory, and diuretic properties. The antioxidant, antimicrobial, anti-carcinogenic, and anti-tumor properties of flavonoids are well known [18]. Flavonoids play a significant role in disease prevention and treatment [19]. Biological function of flavonoids also include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepotoxins, viruses and tumor [20]. Saponins of plants had been usually considered as antibacterial, anti-inflammatory and antitumour activities. 1

CONCLUSION

Tribulus terrestris is popular for its medicinal properties in the folk as well as indigenous system of medicine. Most of the traditional claims of *Tribulus terrestris* have been scientifically validated. Phytochemical screening and analysis will be useful in the presence and quantification of the bioactive principles and subsequently may lead to the drug discovery and development. The results obtained from the present phytochemical and secondary metabolites analysis of the leaves of *Tribulus terrestris* showed the presence of carbohydrate, Flavonoid, tannin, alkaloids, glycosides, Saponin, phenol. Many evidences that were gathered in earlier studies also confirm the identified primary and secondary metabolites to be bioactive constituents which are medicinally valuable. Therefore, extracts from these could be seen as a good source for developing useful drugs. This present study also concludes that the seed of *Tribulus terrestris* can be utilized as an alternative source of useful drugs.

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