Standardization, Extraction and Phytochemical Evaluation of *Tribulus terrestris* Fruits

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ABSTRACT: *Tribulus terrestris* (TT) is a creeping herb which have plenty of medicinal values, used in Ayurveda and folk medicine in India, China and other countries. The fruits are used to treat various conditions in traditional Chinese medicine. Tribulus possess benefits to cure various ailments. The standardization of plants are essential for the identity and quality of crude drugs for further research. To standardize the plant material, few parameters were estimated as recommended by World Health Organization. Dried and sieved powder of *Tribulus terrestris* fruits were put through organoleptic and microscopic examination followed by physicochemical evaluation. Later the plant material was defatted, extracted and subjected to phytochemical screening. The identity and the purity of the plant material was indicated by macroscopic characteristics and the powdered microscopy is used to determine the specific internal structures. The quality of powdered raw material by physicochemical methods and presence of various phytoconstituents by phytochemical analysis reveals the Pharmacognostic standardization with respect to identity, quality and purity of crude drugs.

KEYWORDS: *Tribulus terrestris;* Ayurveda; folk medicine; physicochemical; and phytochemical screening

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INTRODUCTION

Tribulus terrestris (TT) is an herb with two cotyledons that is native to Bharat, western countries, South Africa, and Australia. It belongs to the Zygophyllaceae family, which includes Genus of twenty-five and approximately two hundred and fifty species. TT is a creeping plant that highten up to one metre high off the ground. Tribulus is derived from the Grecian word "tribolos," indicates "spike fruit." They are employed to treat various conditions in Chinese folk medicine (CFM), Ayurvedic medication in Bharat, and historical medicine in Bulgaria [1, 2]. In Telugu, Palleru; In Sanskrit, Gokshur; in English, puncture vine, caltrops; in hindi, it is

Gokharu; In Gujarathi, Nanagokharu; In Tamil, Nerinjil are some of the common names for TT. It is spread out over a large geographic area. It can be found in Sri Lanka, Kashmir, and many places of India. It is the most habitual creeper on road sides and open fields particularly in sandy, dry and hot regions of India, such as West Rajasthan and Gujarat [3]. TT extract contains numerous compounds with diverse biological properties and chemical structures, including Alkaloids, Carboxylic acids, Tannins, saponins and terpinoids [4]. The spirostanol and furostanol saponins like Hecogenin, Neohecogenin, Tigogenin, Neotigogenin, Gitogenin, Neogitogenin, Diosgenin, and Sarsapogenin are rich sources of *Tribulus*. Its extract contains a steroidal saponin, protodioscin, influence endogenous androgen synthesis by elevating the release of luteinizing hormone [1, 5]. The whole herb have a variety of therapeutic properties, including aphrodisiac, anti-inflammatory, antioxidant and anti-microbial activities. TT is typically helps to treat male-infertility and libido. It could be used for immune-modulatory, hepatoprotective, hypolipidemic, anthelmintic, and anti-carcinogenic purposes [6].

In this article we discuss about the standardization, extraction, and preliminary phytochemical evaluation of *Tribulus terrestris* fruits.

MATERIALS AND METHODS

Procurement of Plant material

The Plant material collected from Ulavapadu (Location 15.1667°N 80.0°E), Nellore district, Andhra Pradesh. The Collected raw material was authenticated by Dr. Noorunnisa Begum, Curator, foundation for revitalization of local health traditions, Bangalore-560064. FRLHT Acc. No. 5671. After Authentication, the fruits were processed, shade dried for two months and powdered in mixer grinder, packed and stored at room temperature in air tight container for further study.

Chemicals and reagents

The required chemicals and reagents in this study were purchased from TCI Chemicals Pvt. Ltd. Kurumandapam, Trichy, Tamilnadu, India and orbit scientific products, Hyderabad, Telangana, India.

Standardization and extraction

According to WHO guidelines and other Pharmacopoeial specifications the powdered fruits and extracts were standardized [7]. Organoleptic and microscopic evaluation, physicochemical parameters like presence of foreign matter, moisture content, and ash value were estimated in dry powder. Fluorescence, and extractive values were measured in various solvents [8, 9]. Extraction was performed by soxhlet apparatus using various solvents with change in polarities [10]. Preliminary phytochemical screening was carried out for all the extracts [11].

Macroscopic and microscopic evaluation

The organoleptic characteristics were noted physically with naked eye compared with the monograph. For the microscopic

examinations coarse powder is taken and examined under laboratory microscope [12].

Foreign matter

Foreign matter include bacteria, fungal molds, animal waste, vehicle pollution, insects other than the plant material. The presence of foreign matter in the plant sample were recorded in the form of percentage weight by weight (% w/w).

Moisture content

The proportions of active ingredients exist in the crude sample were dogged on the basis of dry weight. Hence the moisture content must be resoluted. The laid back technique to determine moisture content is to heat the prerequisite amount of drug till it gets a persistent weight at 100 °C. The crude compound which go through physicochemical change with consequent loss of weight at 100 °C, other methods were employed [13].

Ash value

From the air dried sample, the ash (acid insoluble ash, total ash, and water soluble ash) values were resoluted by the method described in Indian pharmacopoeia [14]. Ash value is used to determine the ratio of inorganic materials (calcium, carbonates, magnesium, phosphates, potassium silicates and sodium) exist in the crude compound. To govern the quality and purity of rudimentary substance these values are essential.

Fluorescence analysis

A pinch of Tribulus fruit powder was placed on a glass slide focused under the microscope and observed in day light and UV light. The powdered sample was placed in various reagents and observed for change in color [15].

Extractive values

The amount of active constituents extracted with selected menstruums from accurate quantity of fruit powder determines the extractive value. Precisely weighed fruit powder was treated with solvents like n-Hexane, n-Butanol, ethanol, methanol, and water. The extracts were collected, subjected to rotavap for solvent recovery except water (aqueous) extract [16].

Phytochemical screening

Identification of the plant metabolites in the extract is one of the preliminary tests for phytochemical screening. The presence of alkaloids, carbohydrates, flavonoids, fats, glycosides, proteins, saponins, sterols, and tannins [17-20].

RESULTS

Macroscopic and microscopic evaluation

Tribulus terrestris is a silky hirsute shrub with opposite leaves with equal number of leaflets on both sides of midline with yellow flowers having characteristic odour. The

height of plant is approx. 70 cm when measured. The fruits are small, nearly round shaped with 2-5 sharp spikes consists of seeds within them. Seeds are wet and oily in nature. The spiky fruits appears as cow hoof therefore it is also called as gokshura [3]. Roots appears as slender with bunch of rootlets with cylindrical shape. The macroscopic characteristics of plant, dried fruits and its powder were represented in figure 1. The powdered microscopy contains numerous epidermal trichomes parenchyma cells, polygonal thick-walled cells, epidermal cells, sclerenchymatos cells, fibre sclereids, and few crystals. The structures of few cells were depicted in figure 2.



Figure 1: Macroscopy of *Tribulus terrestris* herb. A: Whole plant of *Tribulus terrestris*. B. Dried fruits of *Tribulus terrestris.* C. *Tribulus terrestris* fruits powder.

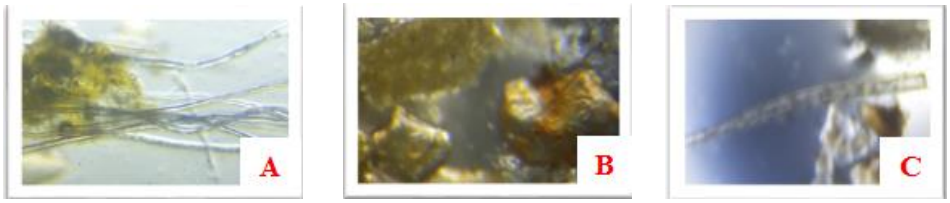


Figure 2: Powdered microscopy of *Tribulus terrestris* fruits. A: Fibre tracheids and Brachy sclereids. B. Calcium oxalate crystals. C. Trichomes.

Physicochemical parameters

Physicochemical parameters like foreign matter, moisture content, and ash values (Total ash, Acid insoluble ash, and Water soluble ash) were indicated in table 1.

Table 1: Foreign matter, Moisture content (loss on drying), ash values (Total ash, Acid insoluble ash, and Water soluble ash).

Physicochemical parameters	(% w/w)
Foreign matter	0.26

Loss on drying	3.84
Total ash	7.36
Acid insoluble ash	1.2
Water soluble ash	4.1

Fluorescence analysis

The change in the color of powdered fruit sample with various solvents were reported in table 2.

Table 2: The change in color under day light and UV light.

TREATMENT	Day light	*Tribulus terrestris* fruit powder under UV	
		Short UV (264 nm)	Long UV (366 nm)
Fruit Powder as such	Pale Brown	Dark Green	Pale Green
Fruit Powder + 1N sodium hydroxide	Dark Green	Pale Green	Greenish Brown
Fruit Powder + 1N sulphuric acid	Greenish Brown	Black	Pale Black
Fruit Powder + 1N hydrochloric acid	Dark Brown	Pale Green	Light Green
Fruit Powder + 1N nitric acid	Yellow Brown	Dark Green	Black
Fruit Powder + acetic acid	Pale Brown	Dark Brown	Greenish Brown
Fruit Powder + 5% iodine	Dark Green	Pale Green	Purplish Green

Extractive values

The extractive values of each extract in individual solvent were expressed in table 3.

Table 3: Extractive values of *Tribulus terrestris* fruit powder in various solvents.

S. No	Solvent	(% w/w)
1	n-Hexane	28.66
2	n-Butanol	15.68

3	Ethanol	9.56
4	Methanol	8.52
5	Water	7.1

Preliminary phytochemical screening

The presence of various phytoconstituents were tabulated in table 4.

Table 4: Phytochemical screening *Tribulus terrestris* fruits.

Tests	Methanolic extract	ethanolic extract	Aqueous extract
Alkaloids	+	++	++
Carbohydrates	++	++	+
Saponins	++	++	-
Steroids	++	++	+
Tannins	+	++	-

(++) Highly present (+) present (-) absent

DISCUSSION

The macroscopic and microscopic characteristics reveals the identity and purity of the plant material. where as the physico-chemical properties are indicted in (% w/w). The presence of foreign matter is 0.26, loss on drying is 3.84, total ash 7.36, acid insoluble ash 1.2 and water soluble ash 4.1. The fluorescence for powder was observed as such, under short and long UV. The extractive values of n-Hexane is 28.66 , for n-Butanol 15.68, Ethanol 9.56, Methanol 8.52 and Water 7.1 and various phytochemicals like presence of alkaloids, carbohydrates, saponins, steroids and tannins were confirmed.

CONCLUSION

Pharmacognostic standardization is a critical component of establishing the precise identity and purity of a basic material. This can be done by evaluating its organoleptic, microscopic, and other parameters. The concurrent study provides many qualitative and quantitative principles of Tribulus terrestris fruits. This standardization will provide referential data on the identity, quality, and purity of the crude drugs, preventing them from being degraded by a similar substandard product.

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Conflicts of Interest

The authors declare no conflict of interest.

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