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Ethnomedicinal and Pharmacological Values of *Tribulus Terrestris* L.: An Important Medicinal Herb of India

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Abstract

Tribulus terrestris, also referred to as "Gokhura," is an annual herb used for medicinal purposes that is a member of the Zygophyllaceae family. It has an extensive history of use in the healthcare systems of both China and India. A concerted effort has been made to examine the literature, collect plant parts from the Mahanadi River areas in Cuttack, Odisha, and analyze the phytochemicals for quality and toxicity against *Artemia salina* L., all while keeping in mind the significance of Gokhura. In 2023, the fieldwork and literature review were completed. According on the findings, many researchers have found various pharmacological values. Different bioactive chemicals were found in different plant parts, based on the results of the experiment. The death % showed highest in all plant parts against *Artemia salina* L. The present study highlights that the population of this important herb is declining and need to restore with local communities. The experimental results also highlight its pharmacological values for further advanced research.

Key words: Local herb, medicinal values, future drugs, restoration

INTRODUCTION

Primary health care systems are based on medicinal plants since ages and used by different tribal communities of the world (Mathur and Sundaramoorthy, 2013). This practice is known as Indigenous Traditional Knowledge (ITK). ITK is base of modern medical systems but unfortunately ITK is a neglected subject. After COVID-19, when world realized the importance of traditional medicines, again started documentation and work on ITK globally. ITK on therapeutic systems reveals number of herbs of India having lot of medicinal values and could be the source for active constituents against several health problems. Among them, *Tribulus terrestris*, popularly known as Gokhuru, an

important medicinal plant of many Indian ayurvedic formulation (Neychev and Mitev, 2005; Shahid et al., 2016; Stefanescu et al., 2020). It is also a part of Dashmularishta even modern and urban people are not able to identify if it is available near their feet. The genus *Tribulus*, comprises about 20 species in the world, of which three species, viz. *Tribulus cistoids*, *Tribulus terrestris*, and *Tribulus alatus*, are of common occurrence in India (Trease and Evans, 2002). The name *Tribulus* comes from the Greek name "*tribolos*" which means spike fruit. The fruits are used in traditional Chinese medicine, in Ayurvedic medicine in India for the treatment of different ailments (Pokrywka et al., 2014). It is a cosmopolitan herb found in Mediterranean, subtropical, and desert climate regions around the world, viz. India, China, Southern USA, Mexico, Spain, and Bulgaria (Chhatre et al., 2014). It is a common weed of the pasture lands, road sides, and other waste places, mainly in hot, dry, and sandy regions including West Rajasthan and Gujarat in India (Kokate et al., 2007). *Tribulus terrestris* is a small prostrate, about 5-10 cm long, 10-30 cm height, hirsute or silky hairy shrub. The leaves are opposite, often unequal, paripinnate; pinnae from five to eight pairs, elliptical or oblong lanceolate (Gupta, 2017). The flowers are yellow in colour. Its carpel fruits are of characteristic, stellate shape, somewhat round-shaped, compressed, five cornered, and covered with princkles of very light-yellow colour. There are several seeds in each crocus with transverse partitions between them. The seeds are oily in nature. When fresh, the root is slender, fibrous, cylindrical, frequently branched, bearing several small rootlets and is of light brown colour. Fruits and roots are mainly used as a folk medicine for the treatment of various ailments. Root occurs in pieces, 7-18 cm long and 0.3-0.7 cm in diameter, cylindrical, fibrous, frequently branched, bearing several small rootlets, tough, woody, yellow to light brown in colour, surface rough due to the presence of small nodules; fracture fibrous; odour aromatic; taste sweetish astringent. The spiky fruit looks like the cloven hoof of a cow and hence, is known as go-ksura (Chhatre et al., 2014). Fruits are faint greenish yellow with spines. They are globose, consisting of five, nearly glabrous, muriculate, wedge-shaped, woody cocci, each with 2 pairs of hard sharp spines, one pair longer than the other. Tips of spines almost meet in pairs together forming pentagonal framework around the fruit (Kevalia and Patel, 2011). Keeping the importance of Gokhuru, an attempt has been made to gather its medicinal values from literature and field survey. An experiment was designed to know the available bioactive compounds from the different plant parts of Gokhuru and evaluate the toxicity against *Artemia salina*.

MATERIALS AND METHODS

Primary data on population and interaction with locals: About 30 km area near Mahanadi River was surveyed in Cuttack district from Mundali and discussed with locals on population and uses of *T. terrestris*. Plant was identified by the authors followed by Flora book (Saxena and Brahmam, 1994).

Collection of plant parts: The plant parts (Leaves, stem, flowers, fruits, and roots) were collected during July 2023 from nearby surroundings of Mahanadi River area of the district Cuttack, Odisha (Plate 1). During the collection of plant samples, we used scissors, globes to protected hands from the sharp spines of fruits.

Processing of plant sample: The leaves, stems, flowers, fruits, and roots of *T. terrestris* were thoroughly washed in tap water and then rinsed with distilled water. Then allowed to dry and powdered through electric blender jar. The powders of different plant parts were stored in air tight containers, protected from sunlight until proceed for analysis (Marandi et al., 2024).

Extraction: Plant extraction was carried out using Soxhlet apparatus and dried till semi-soli mass. Semi-soli mass was kept in refrigerator for experimental works (Kumar et al., 2013; Devi et al., 2023).

Phytochemical analysis: Qualitative analysis of phytochemicals was carried out using standard method (Devi et al., 2023; Marandi et al., 2024).

Carbohydrate: (Fehling's test) 1ml filtrate was added with Fehling's solution A and B, and kept for 5 minutes in water bath. Formation of red precipitation indicates the presence of carbohydrate.

Lipid: (Sudan-III test) 1ml extract was added with 2-5 drops of Sudan-III. The appearance of pink droplet indicates the presence of lipid.

Protein: (Millon's test) 1ml extract was mixed with 2-3 drops of Millon's reagent and kept the solution in water bath for 3 minutes, the pink colour formation indicates the presence of protein.

Amino acid: (Ninhydrin test) 1ml filtrate was added with 5 drops of Ninhydrin solution and kept in water bath till purple colour appeared, denotes the presence of amino acid.

Tannins: (Lead acetate test) 1ml of extract was added to 3-5 drops of 0.1% lead acetate, and the yellowish precipitation indicates the presence of tannins.

Saponins: (Froth test) 1ml of extract was added with 2 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

Flavonoids: 1ml of filtrate was added with 2ml of 10% NaOH solution and 2-3 drops of concentrated sulphuric acid respectively. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

Terpenoids: (Chloroform test) 1ml of extract was added with 1ml of chloroform and then 3-4 drops of concentrated sulphuric acid were added to it. Then a reddish-brown colour ring formation of interface indicates the presence of terpenoids.

Phenolic compounds: (Ferric chloride test) 1ml filtrate was added with 3-5 drops of 1% ferric chloride solution. Then the bluish-black colour formation in the solution indicates the presence of phenolic compounds.

Reducing sugar: (Fehling's test) 1ml filtrate was added with 2 drops of Fehling's solution A and B respectively and kept the solution in water bath for 5 minutes. Then the formation of orange red precipitation indicates the presence of reducing sugar.

Cytotoxicity against *Artemia salina* L.

1% DMSO was taken for the brine shrimp assay to prevent the toxicity of DMSO. 1% DMSO and artificial sea water were used as negative controls. The hatching of eggs was done using artificial sea water made by dissolving commercial marine salt (3.6 g) in distilled water (200 ml). The nauplii hatched within 18-24 hours at 30-35 ^{0}C. Ten larvae of brine shrimps were transferred to each prepared test tube containing extracts of different concentrations (50, 100, 150 and 200 µg/ml) as

per standard methods (Sarah et al., 2017). Survivors were counted every hour for up to 24 hours and then up to 48 hours. The total death and percentage mortality at each concentration level and control were determined and recorded (Sarah et al., 2017; Marandi et al., 2024).

RESULTS AND DISCUSSION

Literature survey

Literature survey revealed that it has anti-inflammatory properties (Chhatre et al., 2014), analgesic activity (Heidari et al., 2007), anticancer potential (Kumar et al., 2009), antidiabetic activity (Li et al., 2002), anticariogenic activity (Oh et al., 2011), anthelmintic activity (Kiran et al., 2011; Deepak et al., 2002), aphrodisiac potential (Kavitha et al., 2012; Adaikan et al., 2001), antispasmodic activity (Chhatre et al., 2014), antiurolithic activity (Aggarwal et al., 2012), central nervous activity (Deole et al., 2011), diuretic activity (Ali et al., 2003; Chhatre et al., 2012), hepatoprotective activity (Kavitha et al., 2011) and larvicidal activity (Singh et al., 2008; Ei-Sheikh et al., 2012).

Plate 1: showing different parts; (a) Flowers, (b) Fruit, (c) Leaves with flower, and (d) stem of *T. terrestris*

Primary Population inventory

30 km areas of Mahanadi River in Cuttack district are covered. The results of the survey revealed that in only 5 locations, authors found the population of *T. terrestris*. Discussion with local fishing community revealed that youth have no idea about the plants but elder people have idea about its medicinal properties.

Traditional therapeutic uses

During survey and interaction with locals, authors found that whole plant decoction is used to treat sexual disorders and urine infections. It was noticed that root of *T. terrestris* is used to make tonic.

Moisture % and Phytochemical screening

Highest moisture % was observed in fruits followed by flowers, leaves, and stem (Table 1). From the result, it was observed that a stem showed lowest moisture percent which indicate a good plant part

for further drug formulation. From the result, it was noticed that stem could be best part to analyse the bioactivity, followed leaves, flowers, and fruits.

Table 1: Moisture content (% weight) estimation in plant parts of *Tribulus terrestris*

Plant parts	Fresh weight (in gram)	Dry weight (In gram)	Moisture %
Leaves	10	2.16	21.6
Stem	10	1.8	18.0
Flower	10	2.2	22.0
Fruit	10	2.4	24.0

Table 2: Bioactive compounds detected in aqueous extract of *Tribulus terrestris* parts

Plant parts	Phytochemical compounds
Leaves	Tannin, saponin, flavonoids, terpenoids, phenolic compounds, steroid, and alkaloids.
Flowers	Saponin, flavonoids, terpenoids, phenolic compounds, reducing sugar, steroids, and carbonyl compounds.
Fruits	Tannin, saponin, flavonoids, terpenoids, reducing sugar, steroid, alkaloids, and carbonyl compounds.
Stem & roots	Tannin, saponin, terpenoids, reducing sugar, steroid, alkaloids, and carbonyl compounds.

Phytochemical screening revealed that in aqueous extract of leaves showed the presence of tannin, saponin, flavonoids, terpenoids, phenolic compounds, steroid, and alkaloids whereas aqueous extract of flowers showed saponin, flavonoids, terpenoids, phenolic compounds, reducing sugar, steroids, and carbonyl compounds. The aqueous extract of fruits showed the bioactive compounds like tannin, saponin, flavonoids, terpenoids, reducing sugar, steroid, alkaloids, and carbonyl compounds and aqueous extract of stem & root showed the presence of tannin, saponin, terpenoids, reducing sugar, steroid, alkaloids, and carbonyl compounds. The above bioactive compounds revealed the pharmacological values of *T. terrestris* (Table 2). It was noticed that fruits and flowers (aqueous) extract showed highest death rate after 8 hours followed by leaves and steam-root (Table 3). It indicates that it could be useful to make drugs against cancer cells.

Table 3: Cytotoxicity analysis of *Tribulus terrestris* against *Artemia salina* L

Plant parts	Concentration (µg/ml)	Initial number of Nauplii	Number of deaths of Nauplii		Death rate after 8 hours (in %)
			After 8 hours	After 24 hours	
Leaves	200	10	8	10	80
	150	10	7	10	70
	100	10	7	10	70
	50	10	6	10	60
Flower	200	10	10	10	100
	150	10	10	10	100
	100	10	8	10	80
	50	10	8	10	80
Fruit	200	10	10	10	100
	150	10	10	10	100
	100	10	8	10	80
	50	10	6	10	60

Stem and root	200	10	8	10	80
	150	10	7	10	70
	100	10	6	10	60
	50	10	6	10	60
Vincristine sulphate	1(mg/ml)	10	10	10	100
Saline	3.5(%)	10	0	0	0

Conclusion

Medicinal plants play an important role in Indian therapeutic systems and provide source for the development of drugs. In this aspect, *T. terrestris* could be a good source as it has diverse bioactive compounds in its plant parts and showed cytotoxicity activity against *Artemia salina*. Further, more advanced experimental works are needed. The population of this herb is declining in wild. Therefore, cultivation and conservation works should be encouraged.

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