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PHARMACOGNOSTICAL EVALUATION OF *TRIBULUS TERRESTRIS* L.

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ABSTRACT: Objective: The present study was designed to evaluate the pharmacognostical and quality control parameters of *Tribulus terrestris* L. whole plant except fruits. **Method:** The plant was collected from the university campus and was got authenticated from NBPGR, Delhi. Various standardization parameters viz. loss on drying, ash values, extractives values, swelling index, foaming index etc. were determined as per the procedure laid down in Ayurvedic Pharmacopoeia of India and WHO Guidelines. Macroscopic and microscopic studies were also performed. **Results:** The present study provided pharmacognostical, physicochemical details, elemental analysis and microbial contamination of the plant which will help in designing the pharmacopoeial monograph. The microscopic studies established that the leaves are of dorsiventral type having single celled trichomes all over the stem and leaves. The plant extracts contained safe pesticides as revealed by the GCMS analysis. The phytochemical screening establishes the presence of alkaloids, carbohydrates, phenol, flavonoids, saponins and phytosteroids. **Conclusions:** The monograph of the plant can be established for pharmacognostical parameters, thereby confirming the credibility of the plant as a medicinal herb.

INTRODUCTION: *Tribulus terrestris* L. (TT) belonging to family Zygophyllaceae, commomnly known as chota gokhru in hindi is a trailing and spreading herb, densely covered with miniature hairs [1, 2]. The preliminary phytochemical study of TT revealed the presence of saponins, flavonoids, glycosides, alkaloids, and tannins [3]. Steroidal saponin, diosgenin and other phytosterols namely sitosterol and stigmasterol are present in roots, stem and leaves [1].

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The chemistry and bioactivity of saponins in TT have been reported due to presence of furostanol and spirostanol saponins namely tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, chlorogenin, ruscogenin and sarsasapogenin. Four sulfated saponins of tigogenin and diosgenin type have also been isolated. Furostanol glycosides including protodioscin and protogracillin are also present. Protodioscin is the most dominant saponin and spirostanol glycosides are present in small quantities in TT. Main flavonoids are about 1.5 times that of main saponins. Several flavonoids that have been isolated from leaves as well as fruits of TT are kaempferol, kaempferol-3-glucoside, kaempferol-3- rutinoside and tribuloside [kaempferol-3-β-d-(6″-p-coumaroyl) glucoside] [4].

Till date numerous pharmacological activities have been studied on different parts of TT plant. It has significant diuretic, aphrodisiac, antiurolithic, immunomodulatory, acetylcholinesterase (ACE) inhibitory, antidepressant, anxiolytic, hepatoprotective, antiinflammatory, antispasmodic, cancer preventive, antibacterial, anthelmintic, larvicidal, anticariogenic and antidiabetic potentials [4].

TT is used in folk medicines as a tonic, aphrodisiac, palliative, astringent, stomachic, antihypertensive, diuretic, lithotriptic, and urinary disinfectant. The dried fruit of the herb is very effective in most of the genitourinary tract disorders. TT has been used for centuries in Ayurveda to treat impotence, genital diseases and sexual frailty. In Bulgaria, the plant is used as a folk medicine for treating impotence.

In addition to all these applications, the Ayurvedic Pharmacopoeia of India attributes cardiotonic properties to the root and fruit. In traditional Chinese medicine, the fruits were used in eye trouble, edema, abdominal distension, emission, morbid leukorrhea and sexual dysfunction. Shern-Nong Pharmacopoeia (the oldest known pharmacological work in China) described its use in depressed liver, mastitis, flatulence, acute conjunctivitis, headache and vitiligo. In Unani medicine, TT is used as diuretic, mild laxative and as a general tonic [5]. This plant is a most important ingredient of an Ayurvedic preparation. Leaf decoction is used as mouth gargle. Leaves increase the menstrual flow and cure gonorrhea. The root is claimed to be stomachic, appetizer, diuretic and carminative. It is used in well-known ayurvedic medicines namely *Gokshuradi Guggul*, *Dashmoolarishtha* and *Amritha Prasa Ghritha* prescribed for several diseases [1, 6].

There are no reports on pharmacognosy and preliminary phytochemistry on the whole plant of TT. Therefore this work on pharmacognosy and phytochemistry on TT was designed to standardize the plant as it grows as a common weed and could be very useful to common people as medicinal herb as well as common nutraceutical. The plant therefore was assessed as useful through the standardized pharmacognostic tools.

MATERIALS AND METHODS:

Collection and authentication: The whole plants of *Tribulus terrestris* L. was collected from the University campus of Guru Jambheshwar University of Science and Technology, Hisar, Haryana in the month of August and October, 2013. The collected plant material was shade dried for about 12-16 weeks at room temperature. All the fruits of plant were removed cautiously. The plant was authenticated by Dr. Anjula Pandey, Principal Scientist at NBPGR, New Delhi. The authentication no is NHCP//NBPGR/2013-9 and a voucher specimen has been kept in the department herbaria for the future references. The dried material was coarsely powdered mechanically with the help of a grinder, passed through 20 mesh sieve and stored in an air tight container till further use.

Preparation of crude extract: The powdered drug (1 Kg) was defatted with petroleum ether (60-80°C) for seven days by cold maceration. The fat exhausted drug was further extracted with ethanol (95% v/v) by soxhlation for 72 h. The extract was concentrated in rotary vacuum evaporator (Heidolph, Germany) to yield greenish black coloured mass. The ethanol exhausted marc was air dried for 24 h at room temperature and then macerated twice in double distillated water for 24 h. The filtered aqueous extract was concentrated on water bath till complete drying and yielded black coloured stiff mass.

Morphological and microscopical studies: The morphological characterization of various parts of TT were done using dissection microscope. Various sensory parameters such as colour, odour, taste, size, shape, texture were studied by the organoleptic evaluation. Fresh plant material was used for the study of anatomical features. The stem, root and leaf were studied by taking hand sections from the fresh plant. These sections were cleared with chloral hydrate solution to remove chlorophyll and stained with phloroglucinol and hydrochloric acid, then mounted in glycerin for the identification of various regions. Microscopical characters were observed using the fine powder and stained with safranin O and fast green and then mounted with glycerin. Microscopic pictures were taken using Motic microscope BA 200 and camera moticam 480 [7, 8].

Physicochemical and preliminary phytochemical screening: Physiochemical values such as the percentage of ash values (total ash, acid insoluble ash and sulphated ash), extractive values as ethanol soluble extractives and water soluble extractives were calculated according to the methods described in the Ayurvedic Pharmacopoeia of India and Indian pharmacopoeia **Table 1** [9, 10]. Phytochemical studies such as qualitative examination were done on the ethanol and aqueous extracts for the identification of the various classes of active chemical constituents, using standard method. Qualitative chemical tests were conducted in order to identify the presence of various phytoconstituents *viz*; alkaloids, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, saponins and proteins **Table 2** [10, 11].

Pesticide residue analysis: Pesticide determination was done by using Shimadzu GCMS-QP2010Ultra (Japan make) and the peaks were matched with the pesticide library. Both ethanol and aqueous extract were diluted with ethanol and filtered with Whatman No. 42 to obtain particle free extract for pesticidal analysis by GC-MS **Table 3, 4**.

Heavy metal analysis: Digested samples were analyzed by Atomic Absorption Spectroscope (AAS) (GBC 932 plus). The instrument was calibrated by using standard solutions of Arsenic, Lead, Cadmium, and Mercury at wavelengths 193.7 nm, 217 nm, 228.8 nm and 253.7 nm respectively. The standard calibration curves were prepared. The instrument was optimized as per requirement and results were obtained in ppm levels [12].

RESULTS:

A) Morphological studies Fig.1

FIG. 1: WHOLE PLANT *TRIBULUS TERRESTRIS* L.

Macroscopy of leaf: Colour of leaves were dark green when fresh and light green on drying. The plant had characteristic odour. The fractures were fibrous and texture was brittle. Whole plant had a slightly sweet taste. Leaves were pinnately compound, hairy, stipuled, oppositely paired, obovate, 3-8 in pair, upto 8 cm long and had complete lamina.

Macroscopy of roots: The roots were creamish to light brown in colour and had tap root and adventitious rootlets. Drug consisted of root, 7-18 cm long and 0.3- 0.7 cm in diameter, slender, cylindrical, fibrous, frequently branched bearing a number of small rootlets, tough, woody and yellow to light brown in colour.

Small nodules present on the root made it rough, fracture was fibrous, odour was aromatic and the taste was sweetish and astringent.

Macroscopy of stem: Colour of stem was dark green when fresh and light green on drying. The plant had characteristic aroma. It was pubescent, rough, length varied between 0.5- 5 feet, prostate, branched and swollen at nodes.

(B) Microscopical Studies:

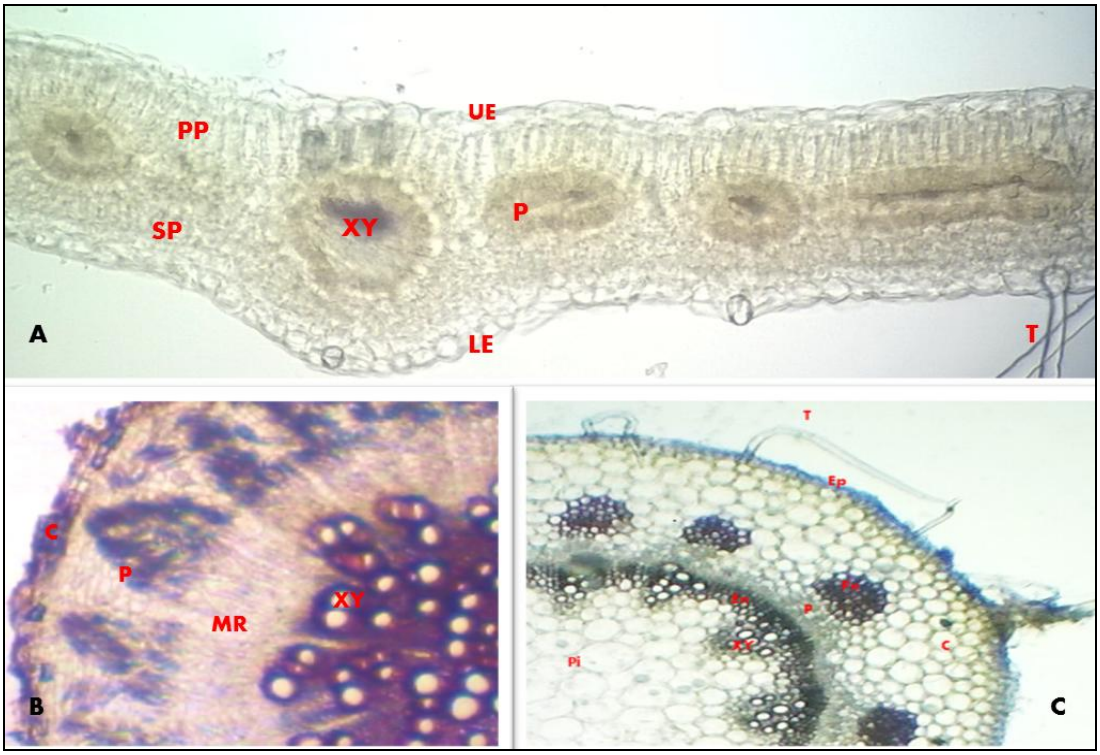

FIG. 2: MICROSCOPY OF PLANT PARTS: A) TRANSVERSE SECTION OF LEAF, B) TRANSVERSE SECTION OF ROOT, C) TRANSVERSE SECTION OF STEM [Abbreviations: **UE**- upper epidermis, **LE**- lower epidermis **PP**- palisade parenchyma, **SP**- spongy parenchyma, **XY**- xylem, **P**- phloem, **C**- cork in root, **c**- cortex in stem, **MR**- medullary rays, **T**- trichome, **Ep**- epidermis, **Pe**- pericycle, **En**- endodermis, **Pi**- pith]

Microscopy of leaf: The leaf was dorsiventral type with a single layer of upper epidermis and thin cuticle. Unicellular trichomes and stomata were present on both upper and lower epidermis. The mesophyll between two epidermal layers was differentiated as palisade and spongy parenchyma. The mesophyll formed of one layer of elongated, cylindrical palisade parenchyma layer at right angle to upper epidermis. Spongy parenchyma constituted of oval and rounded cells near the lower epidermis. Vascular bundles were distributed throughout the leaf blade and their size gets reduced as they approach the leaf margin. Xylem was present towards the upper epidermis and phloem towards the lower. There were bundles of phloem parenchyma present in leaf. Collenchyma was absent **Fig. 2 A**.

Microscopy of roots: Transverse section of primary roots showed a layer of epidermis followed by 4-5 layers of thin-walled parenchymatous cortex, endodermis was distinct, pericycle encloses diarch stele, in mature root the cork was 4-6 layered. The inner zone was parenchymatous, phloem rays were distinct. Wood composed of vessels, parenchyma traversed by medullary rays, vessels scattered, arranged in singles or doubles towards inner side, in groups of three to four on outer side, xylem parenchyma rectangular or slightly elongated with simple pits and reticulate thickening, medullary rays heterogenous, 1-4 cells wide [9] **Fig. 2 B**.

Microscopy of stem: The stem had single layer of epidermis and hypodermis. Stem is hairy due to presence of numerous unicellular trichomes on its surface. Cortical cells were present in abundance but no cambium was present. A layer of endodermis was observed.

Phloem was present towards outside and primary and secondary xylem were present in a fixed pattern. The central portion was composed of rounded or polygonal thin walled parenchymatous cells without intercellular spaces **Fig. 2 C**.

Microscopy of powder: The powder had two types of xylem vessels namely spiral and pitted. Starch grains with hilum, phloem fibers, rosette shaped calcium oxalate crystals and unicellular trichomes were present in abundance **Fig. 3 A-G**.

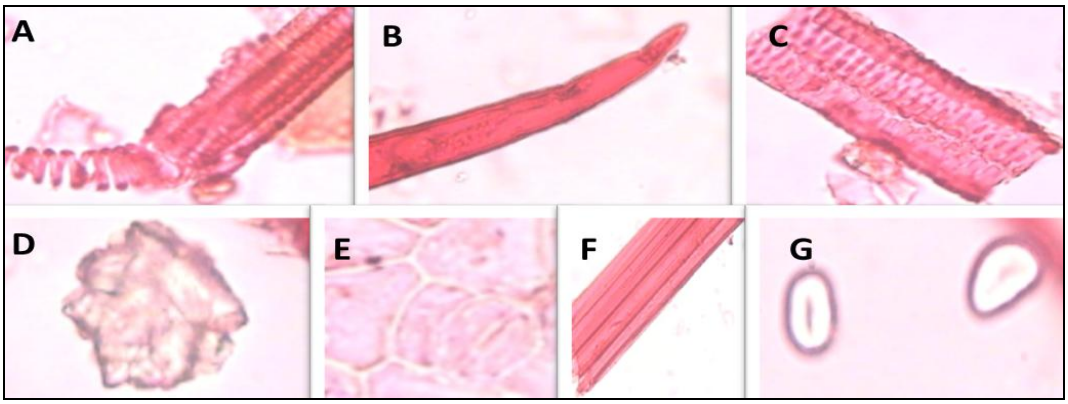

FIG.3: MICROSCOPY OF POWDER: A) SPIRAL XYLEM VESSEL, B) UNICELLULAR TRICHOME, C) PITTED XYLEM VESSEL, D) CALCIUM OXALATE CRYSTAL (ROSETTE SHAPE), E) PARACYTIC STOMATA, F) PHLOEM FIBRES, G) STARCH GRAIN WITH HILUM

TABLE 1: PHYSICOCHEMICAL ANALYSIS

S. No.	Parameters	Values
1	Foreign matter	0.8 ± 0.03 %
2	Ash values – Total ash	9± 0.13%
3	Ash values – acid insoluble	1.8±0.27 %
4	Ash values – sulphated ash	12.3±0.36 %
5	Extractive values ethanol soluble	56.6±0.41 mg/g
6	Extractive values water soluble	134.0±5.7 mg/g
7	Moisture content (Gravimetric method)	30.0±0.71 mg/g
8	Swelling index	1±0.07 ml/g
9	Foaming index	Less than 100

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOL AND AQUEOUS EXTRACTS

Plant Constituents / Reagent used	*Tribulus terrestris* L.	
	EE	*AE*
Alkaloids		
Dragendorff's reagent	+	+
Wagner's reagent	++	++
Hager's reagent	++	++
Mayer's reagent	++	+
Carbohydrates		
Molisch's Test	–	+
Fehling's reagent	++	–
Benedict's reagent	++	–
Barfoid test	+	–
Selwinoff's (Resorcinol) test	++	–
Phenolic compounds and tannins		
Ferric chloride test	++	++
Lead acetate test	++	++
Dilute Iodine	++	++
Flavonoids		
Ammonia test	++	+
Shinoda test	++	+
Vanillin-HCl test	++	+
Proteins and free amino acids		
Biuret test	++	–

Xanthoprotein test	–	+
Ninhydrin test	++	+
Saponins		
Frothing test	+	+
RBC haemolysis test	+	+
Phytosterols		
Liebermann-Burchard's test	+	–
Salkowaski reaction	+	–

EE= Ethanol extract; AE= Aqueous extract; ++= present; += slightly present; – = absent

(C) Pesticide Analysis:

TABLE 3: PESTICIDES IDENTIFIED THROUGH GCMS IN TT ETHANOL EXTRACT

S. no No	R.Time	Area%	Name
1	4.441	0.50	Pentoxazone
2	5.134	1.33	Oxamyl
3	9.500	2.97	Amitrole
4	12.246	0.38	Dimepiperate
5	13.750	53.21	Aldicarb
6	15.330	4.64	Triadimenol
7	15.570	3.60	Demeton-S
8	16.743	2.25	Prohydrojasmon-2
9	17.009	12.44	Prohydrojasmon-2
10	17.175	0.82	Allethrin
11	17.246	2.31	Cinerin II
12	17.426	0.90	Demeton-S
13	19.134	0.22	Aldicarb
14	19.675	0.40	Propamocarb
15	19.831	0.42	Jasmolin I
16	20.705	0.39	Aldicarb
17	21.351	0.59	Cinerin I
18	21.483	0.11	Aldicarb
19	21.598	7.98	Jasmolin I
20	22.339	0.25	Aldicarb
21	31.038	0.43	Jasmolin II

TABLE 4: PESTICIDES IDENTIFIED THROUGH GCMS IN TT AQUEOUS EXTRACT

S. no.	R.Time	Area%	Name
1	10.572	0.60	Aldicarb
2	12.964	0.63	Methoprene
3	15.095	13.44	Aldicarb
4	16.079	0.28	Aldicarb
5	16.766	11.07	Jasmolin I
6	16.819	47.64	Jasmolin I
7	17.025	7.61	Aldicarb
8	17.211	5.62	Jasmolin I
9	17.598	6.77	Prohydrojasmon-1
10 10	18.790	0.80	Aldicarb
11	20.418	2.04	Aldicarb
12	22.020	1.06	Aldicarb

(D) Elemental analysis: The Atomic Absorption Spectroscopy study showed the presence of cadmium, lead, arsenic, and mercury in aqueous and alcoholic extracts of plant below the WHO permissible limits and therefore safe to use.

DISCUSSION: The quality control of crude drugs and herbal formulations is important in justifying their acceptability in modern system of medicine. Major problems faced by the herbal drug industries are non availability of rigid quality control profile for herbal material and their formulations. According to world health organization (WHO), the macroscopic and microscopic determination of the plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [7, 11] . A pesticide residue is any specified substance in food, agricultural commodities or animal feed resulting from the use of a pesticide. Gas Chromatography is recommended as the principal method for the determination of pesticide residues. These methods may be coupled with mass spectrometry (MS) [12].

The pesticides present in ethanol and aqueous extracts are namely Aldicarb, Prohydrojasmon-2, Jasmolin I, Jasmolin II, Cinerin I, Cinerin II etc. These all are safe to human beings but repells and kills helminths, termites and are good and safe insecticides used in fields. Contamination of medicinal plant material with arsenic and heavy metals can be due to many causes including environmental pollution and traces of pesticide.

In the present work the macroscopic and microscopic studies of whole plant of TT were carried out. The results of macroscopic evaluation are useful for distinguishing it from its substitutes and adulterants. Microscopic evaluation allowed more detailed examination of crude drug and enabled to identify the organised structural features such as epidermis, starch grains and parenchymatous cells [13]. The moisture content of a drug has to be minimised in order to prevent decomposition of crude drug either due to chemical change or due to microbial contamination. The moisture content was 30±0.71 mg/g. Thus we observed that the plant was very dry and fracture was fragile.

The microscopic characters, quantitative analysis, and physicochemical parameters studied can be used to test the purity of this drug. Since these parameters studied are constant and any change in these values indication of substitution and adulteration. The preliminary phytochemical analysis revealed the presence of carbohydrates, saponins, phenols, tannins, phytosterols and flavonoids. For instance steroids, terpenoids, flavonoids, saponins, tannins and alkaloids have anti-inflammatory effects. Flavonoids, tannins and alkaloids have hypoglycemic activities. Saponins possess hypocholesterolemic and antidiabetic properties and also the terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids and triterpenoids showed the analgesic properties. The steroids and saponins are responsible for central nervous system activities [14–17].

CONCLUSION: As there is no detailed pharmacognostic anatomical work on TT whole plant is reported. Therefore present work is taken up in the view to completely standardize the herb to establish the authenticity of TT and can possibly help to differentiate the drug from its other species. The information gathered from above studies could be of value in the preparation of the herbal monograph.

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