

Standardization of Fruit of *Tribulus terrestris* Linn.

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Different parts of *Tribulus terrestris* Linn. are highly prized remedy amongst the people of India. Since ancient period the fruit is used as demulcent, diuretic, antispasmodic and aphrodisiac. Fruits have been identified by their macroscopic and microscopic characters, cell contents, behaviour of powdered drug with different reagents and preliminary phytochemical analysis.

Key Words: *Tribulus terrestris* Linn.

INTRODUCTION

Tribulus terrestris Linn. (Gokhru) is a herbaceous plant belonging to the family Zygophyllaceae. Different parts of the plant, viz., root, leaf and fruit are extensively used in the Indian system of medicine since ancient period. An infusion prepared from fresh leaf and stem is a highly prized remedy amongst the people of Southern India in gonorrhoea and dysuria. The juice of the fruit is an emmenagogue¹⁻⁵.

Pharmacognostic reports on the root and fruit of the plant are very few and fragmentary^{6,7}. As pharmacognostic screening of the crude drug is essential for identification of the commercial sample, the same has been undertaken to establish the identifying characters for prevention of admixtures and adulterants in the preparation of Ayurvedic formulation. *T. terrestris* is identified as the smaller variety while a large variety equated with *Pedaliium murex*. Linn. (Pedaliaceae) is often used as a substitute for the drug.

EXPERIMENTAL

The plant is widely distributed throughout India up to 11000 ft. *T. terrestris* fruits were procured locally from Modinagar market and identified by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

Macroscopic and microscopic studies were made from free hand. Cell structures of the hard tissues were made by macerating the tissues in conc. HNO₃.

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Powdered drugs were prepared by crushing the fruits in electric grinder. Behaviour of powdered drugs was studied by treating with different chemical reagents. Non-protoplasmic cell contents were studied by treating the sections with chemical reagents. Stains were used and finally mounted either in 50% glycerine or in a mixture of 250% chloral hydrate solution and 50% glycerine solution in the proportion of 9:1. Foreign organic matter, moisture content, ash and extractive values, physical data on fruit of *T. terrestris* Linn. were estimated⁸. Preliminary investigations on fluorescence behaviour of ethanol extracts under long (365 nm) and short (257 nm) UV radiation were also studied.

Macroscopic characters: The fruit is pedicellate, globose, 1.3 cm in diameter, 0.8 cm in thickness, possessing five woody, densely hairy, spiny cocci. Each coccus possesses two large sharp, pointed, rigid spines directed towards the apex. The other two smaller, shorter spines are directed downwards. Tips of spines almost meet in pairs together forming pentagonal framework around the fruit. Outer surface of the schizocarp is rough, yellowish, odour faintly aromatic and slightly acrid in taste. Seeds more or less elliptical, tapering at one end, measuring 1.5 × 3.0 mm. seeds several in each coccus, with transverse partitions between them (Fig. 1).

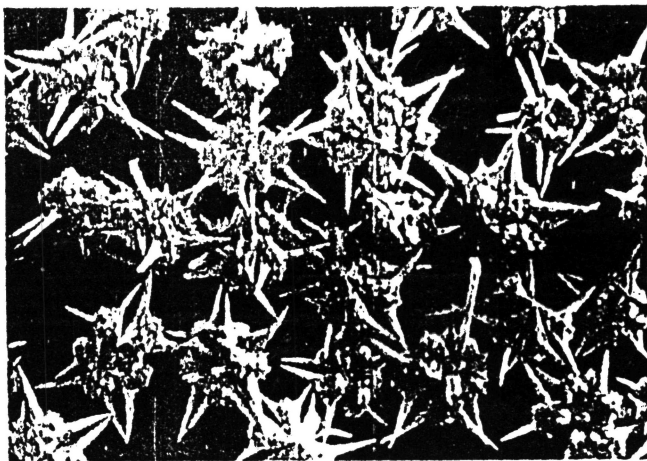


Fig. 1. Fruits of *T. terrestris* Linn

Microscopic Characters: Fruit is very hard, studies are made from macerated tissues as studies from sectional view are not possible. The pericarp is differentiated into epicarp, mesocarp and endocarp. Outer surface of the epicarp is surrounded by non-glandular trichomes. The parenchymatous mesocarp is 6–10 layers thick which embeds calcium oxalate crystals. The sclerenchymatous endocarp is 3–4 layers thick and the cells are compact containing prismatic crystals of calcium oxalate. Fruits are pentalocular, vessels have simple pits and some vessels show helical thickenings. Fibres are lignified, linear, long with tapered ends (Figs. 2 and 3).

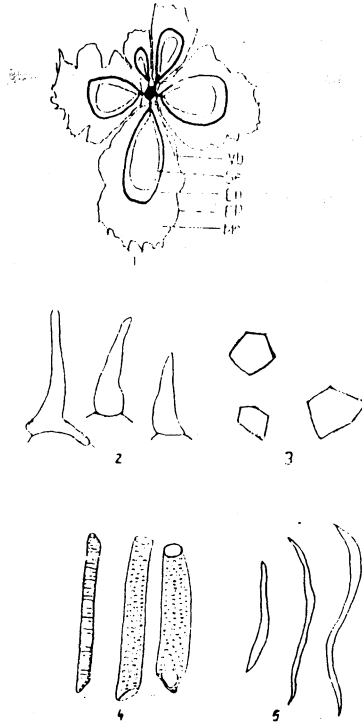


Fig. 2. Microscopic characters of fruit of *T. terrestris* Linn.: (1) T.S. of fruit (diagrammatic), (2) Non-glandular trichomes $\times 180$, (3) Prismatic crystals of calcium oxalate $\times 720$, (4) (i) Vessel showing helical thickening $\times 367$, (ii) Pitted vessels $\times 367$, (5) Fibres $\times 92$

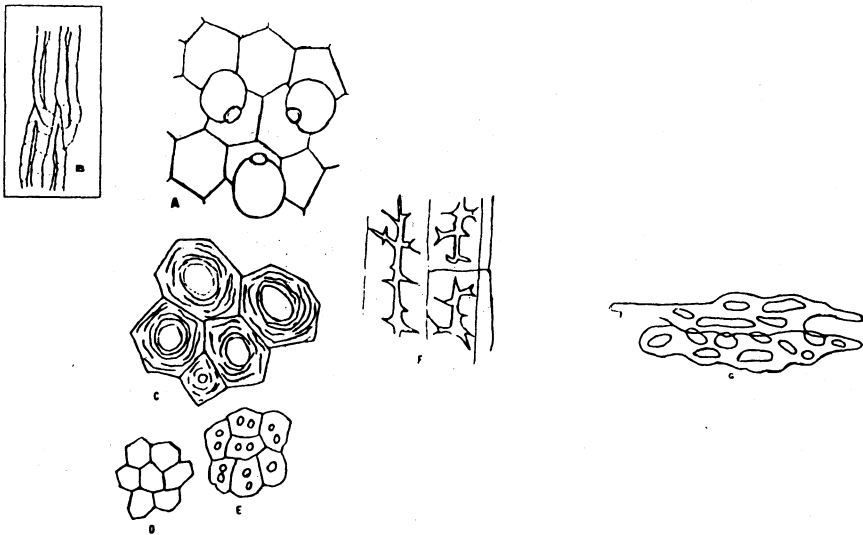


Fig. 3. Microscopic characters of fruit of *T. terrestris* Linn.: (A) Epidermal cells and glands, (B) Bundles of fibres, (C) Cells of outer integument, (D) Cells of inner integument, (E) Endosperm cells with oil drops, (F) Part of sclerenchyma fibres, (G) Reticulated fibres

Physical Constant Values: Foreign organic matter 1.662%; loss on drying 10.10%; total ash 12.79%; acid-insoluble ash 0.97%; sulphated ash 2.07%; water-soluble ash 5.79%; ethanol-soluble extractive 1.862%; water-soluble extractive 16.8%; petroleum ether-soluble extractive 1.018%; chloroform-soluble extractive 1.26%; volatile oil content very small quantity; fluorescent analysis very faint fluorescence in short and long UV light.

Cell Contents: Fats and oil present in the form of globules in the thin-walled cells of the seed; when treated with conc. HCl fat globules are liberated.

REACTION OF POWDERED DRUG WITH DIFFERENT REAGENTS

| | |
|-------------------------------------|---|
| Water | Powder settles at the bottom producing colourless turbid solution with very little frothing on the surface. |
| 5% KOH | Powder settles at the bottom producing brown colored turbid solution. |
| Dil. HCl | Powder settles at the bottom producing very faint lemon yellow tinted solution. |
| Dil. H ₂ SO ₄ | — do — |
| Dil. HNO ₃ | — do — |
| FeCl ₃ solution | Light brown precipitation takes place. |
| Dragendorff solution | Orange brown coloration and precipitation. |
| KI and I solution | Light orange brown turbid solution. |

Preliminary photochemical analysis: Qualitative examination of the various solvent extracts of fruits indicates the presence of alkaloid, fixed oil, lignin, resin, traces of glycosides, protein, tannins, reducing sugars, sterols and an essential oil⁹.

Thin-layer chromatography: 5.0 g sample of powdered fruit was refluxed for 1 h with 50 mL chloroform and filtered. The marc was refluxed for 1 h with 50 mL methanol and filtered. The filtrate was evaporated to dryness under vacuum. 50 mL of 2 N HCl was added to the residue and refluxed for 1 h 1.0 g sodium carbonate was added after cooling the solution and extracted with three successive quantities of 20 mL of chloroform. Combined chloroform layers were washed with water and evaporated to dryness under vacuum. The residue was dissolved in 2 mL of chloroform to be used as test solution.

Test solution and reference solution (1 mg diosgenin in 4 mL methanol) were applied on silica gel G plate, using toluene : ethyl acetate (8 : 2) as solvent system, visualized the spots by spraying the plate with anisaldehyde sulfuric acid reagent and heated at 120°C for 10 min.

A yellowish green spot (R_f 0.29) corresponding to diosgenin was observed in both test and reference solution tracks. Other yellowish green spots (R_f 0.13 and 0.84), prominent violet spots (R_f 0.91, 0.53, 0.43, 0.34 and 0.21) and a dark blue spot (R_f 0.14) were also observed in the test solution¹⁰.

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