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Pharmacognostic characterization and comparison of fruits of *Tribulus terrestris* L. and *Pedalium murex* L.

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ABSTRACT

Tribulus terrestris and *Pedalium murex* are two well-known medicinal plants. Fruits of these plants are used for treating various urinary disorders like calculus formation. In Ayurveda they are known as *Gokshura* and *Brihat gokshura* respectively. In the present paper the fruits of *T. terrestris* and *P. murex* were compared morphologically, anatomically and phytochemically to evolve anatomical and chemical profiles for quality standardization. Reported pharmacological activities of the two fruits were also compared for justifying the use of *P. murex* fruits as a substitute. Main differences were observed in the habit, shape and structure of the fruits. In *T. terrestris*, the fruit is covered with hairs and it is five angled with five segments and a pair of spines from each segment, whereas it is four angled with four spines in *P. murex*. In the TLC profile the two samples showed significant variations and some resemblance. In *P. murex*, several additional bands were noticed in the profile and some bands were seen missing or seen very faint. The presence of common bands in two plants revealed that the methanolic extract of fruits of *P. murex* and *T. terrestris* have some identical chemical constituents and it was also noticed that the intensity of these similar bands were more in *T. terrestris* when compared to *P. murex*. Similar pharmacological activities especially the antilithiatic activity throws light towards the possibility of substitution.

Keywords: *Tribulus terrestris*, *Pedalium murex*, *gokshura*, *brihat gokshura*, physico-chemical, phytochemical, umbelliferon.

1. Introduction

Tribulus terrestris and *Pedalium murex* are the two source plants of the reputed ayurvedic drug *Gokshura*. Fruits of *T. terrestris* are the genuine source of the drug *Gokshura* whereas the fruits of *P. murex*, which is also known as *brihat gokshura* is available in the market as adulterant or unauthorized substitute. Traditionally *Gokshura* fruits are used in Ayurvedic formulations for treating urinary disorders, calculus formation, impotence and other urinary tract infection issues^[1, 2]. According to Ayurveda, the fruits of *P. murex* is also considered to be demulcent and diuretic, antispasmodic, aphrodisiac, improves appetite and useful in strangury, urinary discharges, vesical calculi, cough, asthma, skin diseases and heart trouble. It purifies blood and removes stone in the bladder^[3, 4]. *T. terrestris* is an annual, prostrate hairy herb with many slender spreading branches with opposite, pinnately compound leaves, bright yellow flowers on slender hairy stalks. The plant is distributed throughout India in warm and hot places. It is a common weed of waste places and road sides, chiefly in hot dry or sandy localities. *P. murex* is an erect, glabrous annual herb with simple, opposite or sometimes alternate, petiolate slightly broader leaves. Flowers are yellow, solitary on short, glandular pedicels. It is distributed in Ceylon-Tropical Asia. In India it is seen throughout the warmer parts of the country. Dickison reported that the pharmacognostic study of herb, spice and drug plants involves the microscopic evaluation of plant tissues in sectional & powder form and histological characterization of these plants refers to the minute structure and arrangement of cells and tissues^[5]. In this paper, the fruits of *T. terrestris* and *P. murex* were compared morphologically, anatomically and phytochemically to evolve anatomical and chemical markers for quality standardization. Reported pharmacological activities of the two fruits were also compared.

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2. Materials and methods

2.1 Plant materials

Fruits for the study were collected from their natural habitat. Fruits of *T. terrestris* were collected from different localities of Coimbatore district of Tamilnadu and *P. murex* were collected from Palakkad district of Kerala.

2.2 Method of study

Anatomical: All the materials for anatomical study were fixed in Formaldehyde-Acetic acid-Alcohol (FAA) mixture. Hand sections were taken and histological and histochemical staining was carried out as per standard methods^{16, 71}. The sections were stained with safranin for general studies and Sudan black for oil, iodine for starch, Ferric chloride for tannin and phloroglucinol and Con. HCl for lignin. Powder characters of the two fruits were also compared. Photomicrographs were taken using 'Canon Photospot G3 camera attached to the Ziess microscope.

Phytochemical studies: Roots were shade dried and coarsely powdered. 5gm of powdered roots were extracted with methanol. The extracts were filtered, concentrated and made up to 10 ml. These solutions were used for TLC profiling. Physico-chemical studies were also carried out and compared.

Test solution: 5 g accurately weighed powdered fruit of each plant

were extracted in methanol by refluxing consecutively for a duration of 8 h. Filter the extract and remove the solvent under reduced pressure. Dissolve the residue in methanol in a 10 ml volumetric flask and make up the volume.

Standard solution: Dissolve 10 mg of Umbelliferon in 10 ml of chloroform.

Solvent system: *n*- Hexane: Acetone (7:3)

Procedure: Applied 5 µl of test solutions and 3 µl of standard solution on a pre-coated silica gel 60 F₂₅₄ TLC plate (E. Merck) of uniform thickness of 0.2 mm. Develop the plate in the solvent system in a twin trough chamber to a height of 8 cm.

Visualization: Sprayed the plate with Anisaldehyde-sulphuric acid reagent and heat the plate at 105 °C till the coloured bands developed. Noted the R_f value and colour of the resolved bands

3. Results & Discussion

3.1 Macro and microscopic characters: Macroscopic (external morphology), organoleptic and anatomical characters of fruits of *T. terrestris* and *P. murex* were compared and fruits exhibited a large extent of variation in shape and structure (Table 1).

Table 1: Comparative Pharmacognostic characters of fruits of *T. terrestris* and *P. murex*

Characters observed	<i>T. terrestris</i>	<i>P. murex</i>
Macroscopic characters	Five ribbed or angled, more or less spherical in structure; covered with pubescent hairs, with five pairs of prominent short stiff spines; ripe fruit separates into five segment, starting from its middle (Figure 1 A & B)	Fruit a drupe 4 angled Indehiscent and hard; pyramidal to ovoid with a sharp mucronate spine bluntly with 4 sharp spreading spines with rim of calyx; pedicel short curved; colour pale yellowish brown; mucilaginous (Figure 1 C & D).
Organoleptic characters	Taste, slightly astringent.	Somewhat sweet in taste and no characteristic odour.
Anatomical characters	TS of fruit is pentagonal or star shaped in outline Mesocarp wide and traversed with vascular bundles and stone cells Large calcium oxalate crystals abundantly present, Oil globules present abundantly (3.70%) (Figure 2 G & H) 5 cocci and each cocci containing four or more seeds (Figure 2A),	TS of fruit is somewhat quadrangular in shape Mesocarp is traversed with fibrous sclereids Very few and small cluster crystals of calcium oxalate in the mesocarp Oil globule comparatively less (1%) (Figure 2 O & P) Fruit 2 seeded (Figure 2. J)
Powder characters	Powder is greyish brown and oily consists of fragments of trichomes of various sizes and shapes; isolated or groups of thick walled sclereids from the endocarp and stone cells from the mesocarp, prismatic and cluster crystals from mesocarp; fragments of fibres (Figure 3A-D)	Powder is dark brown, oily fragments of epicarp in surface view; sclereids of mesocarp and testa, inner layer of testa, endosperm cells with oil globules and epicarp of calyx containing colouring material and sclereids from the spine (Figure 3E-H).



Fig 1: A *Tribulus terrestris* L. – habit; B. Dried fruits of *T. terrestris*; C. *Pedalium murex* L. – habit; D. Dried fruits of *P. murex*

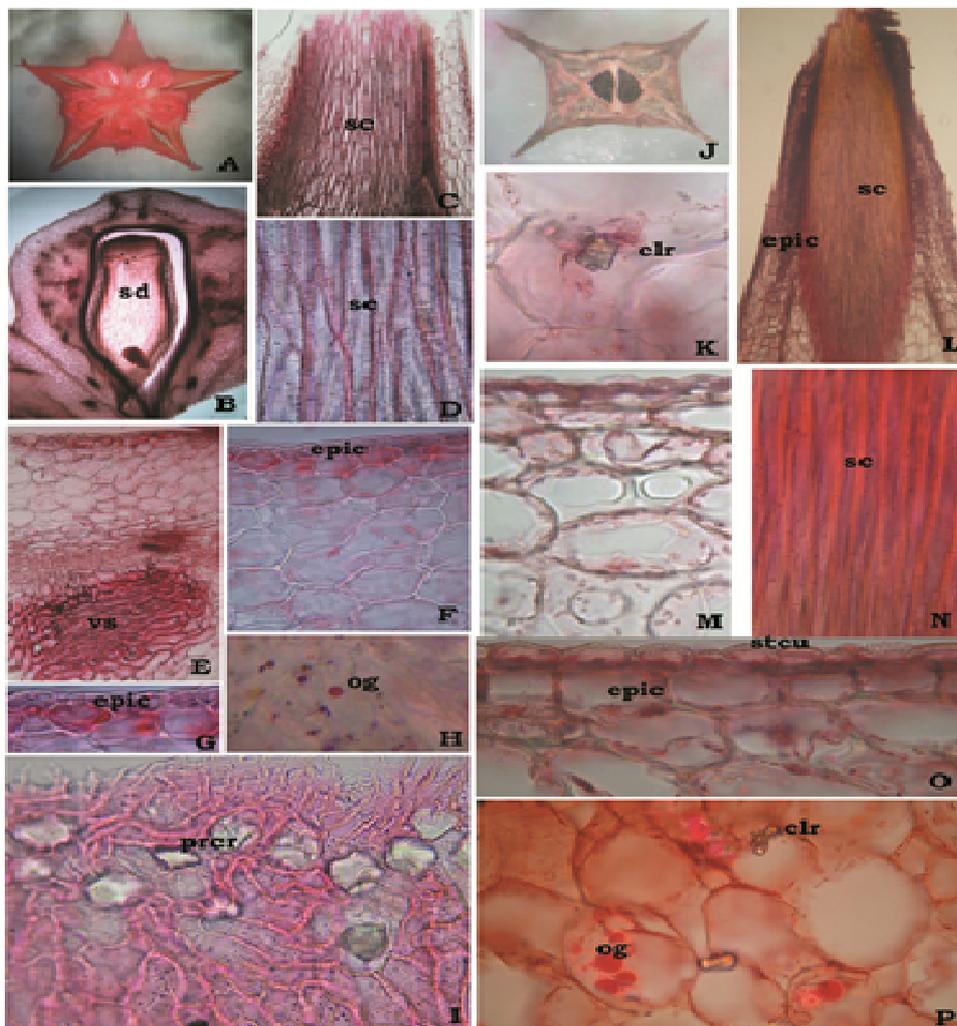


Fig 2: Comparative microscopy of *T. terrestris* and *P. murex* fruits A-I. TS of *T. terrestris* fruit. A. TS of fruit entire x 10; B. Portion of the fruit enlarged showing the seed inside x 40; C. TS of spine x 100; D. Sclereids of spine x 400; E. TS of mesocarp with vascular traces x 200; F. Outer portion of the mesocarp x 400; G. Epicarp x 400; H. Mesocarpic cells with oil globule x 400; I. Sclereids of the endocarp containing crystals of calcium oxalate x 400; J-P. TS of *P. murex* fruit; J. TS of fruit entire x 10; K. Mesocarpic cell containing crystals of calcium oxalate x 400; L. TS of spine x 100; M. Outer portion of mesocarp x 400; N. Sclereids of the spine x 400; O. Epicarp with striated cuticle x 400; P. Mesocarpic cells with oil globules and crystals of calcium oxalate x 400. clr, cluster crystals of calcium oxalate; epic, epicarp; og, oil globule; prcr, prismatic crystals of calcium oxalate; sc, sclereids; sd, seed; stcu, striated cuticle; vs, vascular strand.

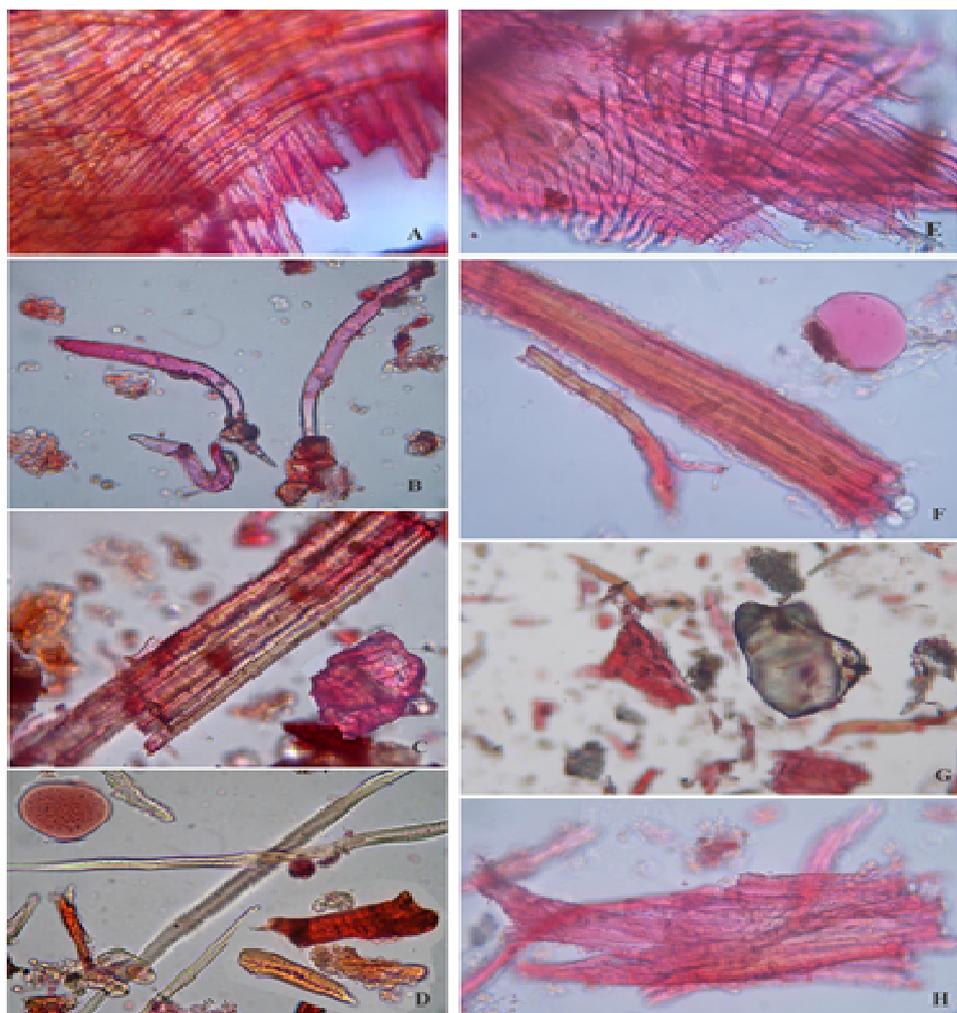


Fig 3: Comparative powder microscopy of *T. terrestris* and *P. murex* fruit. A-D. Powder characters of *T. terrestris* fruit. A. Fibres of testa in surface view; B. Trichomes of various sizes; C. Groups of sclereids from spine and large prismatic crystals; D. Fibres and stone cells of testa and oil globules; E-H. Powder characters of *P. murex* fruit. E. Fibres of testa in surface view; F. Oil globules from mesocarp and groups of sclereids from spine; G. Prismatic crystal and fragments of trichomes; H. Fibres from mesocarp.

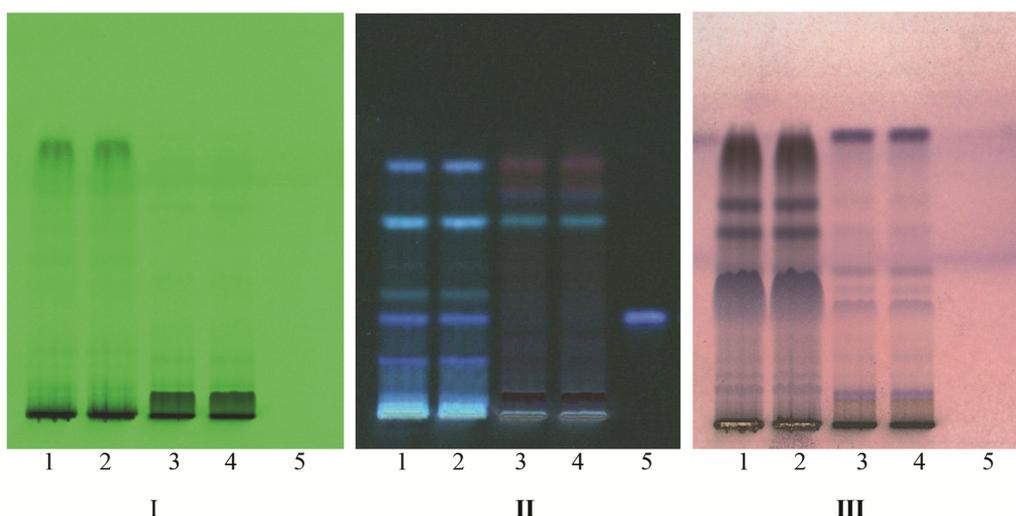


Fig 4: Comparative TLC profile of methanol extract of *T. terrestris* and *P. murex* fruits I- Visualized under UV 254 nm; II- under 366 nm; III- Derivatized with ANS reagent. 1, 2- *T. terrestris* fruit; 3, 4- *P. murex* fruits; 5- Umbelliferon as standard.

3.2 Preliminary phytochemical screening: Preliminary phytochemical screening revealed the presence of phenolic compounds and flavonoids. From the study it was observed that the

phenolic and flavonoid contents are high in *P. murex* which is 31 mg Ega and 0.843 mg Eq (Table 2).

Table 2: Total phenolic and flavonoid content of fruit powder of *T. terrestris* and *P. murex*

Total phenolic and flavonoids	<i>T. terrestris</i>	<i>P. murex</i>
Total phenolic content	28 mg	31 mg
Total flavonoids	0.785 mg	0.843 mg

3.3 Physico-chemical parameters: Physico-chemical parameters such as moisture content, water soluble and alcohol soluble extractives, ash value and acid insoluble ash of fruit powder of *T. terrestris* was determined and compared with that of *P. murex*. From the comparison it was seen that the moisture content was high in *T. terrestris* and it was 10.54%. Extractive values such as water

soluble and alcohol soluble was high in *P. murex*. The values of water soluble and alcohol soluble extractives of *P. murex* are 10.95% and 5.47% respectively. The total ash and acid insoluble ash was high in *T. terrestris* and they were 3.28 % and 0.12% respectively (Table 3).

Table 3: Comparison of physico-chemical parameters of fruits of *T. terrestris* and *P. murex*

Physico-chemical parameters	Values %	
	<i>T. terrestris</i>	<i>P. murex</i>
Moisture content	10.54	7.95
Water soluble extractive (w/v)	14.85	19.95
Alcohol soluble extractive (w/v)	4.98	5.47
Total ash (w/w)	3.28	2.96
Acid insoluble ash (w/w)	0.57	0.12

3.4 Phytochemical studies: The characteristic finger print of the TLC chromatograph can be used as a marker for the quality evaluation of particular sample. The characteristic spots were obtained in TLC profiles of methanol extract of both the fruits and the marker compound, umbelliferon. R_f values of the prominent bands were determined. In the TLC profile the two samples showed significant variations and some resemblance. Several additional bands were noticed in the profile in both the fruits and some bands were seen missing or seen very faint. Common bands were obtained at R_f 0.64 under UV254 nm, at R_f 0.31 (corresponding to marker compound umbelliferon), R_f 0.61 and R_f 0.78 under 366 nm. This indicated that the methanolic extract of fruits of *P. murex* and *T. terrestris* species have some identical chemical constituents and it was also noticed that the intensity of these similar bands were more in *T. terrestris* when compared to *P. murex* (Figure 4 & Table 4).

3.5 Comparison of pharmacological activities: The reported pharmacological activities of two fruits were compared. The reported pharmacological activities of *T. terrestris* are antibacterial^[8], antifungal^[9], antihypertensive^[10], anthelmintic^[11], antidiabetic^[12], antilipidaemic^[13], diuretic^[14], nephroprotective^[15], reno-protective^[16] and anti-urolithiatic^[17]. The reported pharmacological activities of *P. murex* are anti-hyperlipidemic^[18], anti-nephrolithiatic^[19], antibacterial^[20], anti-oxidant^[21] and hepatoprotective^[22].

4. Conclusions

In the present paper an attempt has been made to compare the two *gokshura* in all aspects such as morphological, anatomical, physico-chemical parameters and phytochemical studies such as TLC profiling. The comparative study of these two species is reported here for distinguishing the two. Main differences were observed in the habit, shape and structure of the fruits. Phytochemical characters also showed difference, but some similarities were also observed. The characteristic finger print of the TLC chromatograph can be used as marker for the quality evaluation of particular sample.

In classical texts the fruits of *P. murex* are mentioned as a substitute but in practice it is considered as an adulterant of *T. terrestris*.

Based on the pharmacognostical, pharmacological and phytochemical comparison author has the opinion that the genuine *gokshura* can be equated with the fruits of *T. terrestris*. However antilithiatic property of *P. murex* has to be taken into consideration because activity of *Gokshura* is reported as *asmari* (breaking calculus) in classical texts. Hence the present study justifies the use of *P. murex* fruits as a substitute of *T. terrestris*.

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