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

Jayanthy A, Deepak M, A. B. Remashree

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, hytochemical, 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. Dickson 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.
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 [6, 7]. The sections were stained with safranine for general studies and Sudan black for oil, iodine for starch, Ferric chloride for tannin and phloroglucinol and Conc. HCl for lignin. Powder characters of the two fruits were also compared. Photomicrographs were taken using 'Canon Photospot G3 camera attached to the Zies 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 precoated 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 Rf 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>
<thead>
<tr>
<th>Characters observed</th>
<th><em>T. terrestris</em></th>
<th><em>P. murex</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macroscopic characters</strong></td>
<td>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 &amp; B)</td>
<td>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 &amp; D).</td>
</tr>
<tr>
<td><strong>Organoleptic characters</strong></td>
<td>Taste, slightly astringent.</td>
<td>Somewhat sweet in taste and no characteristic odour.</td>
</tr>
<tr>
<td><strong>Anatomical characters</strong></td>
<td>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 &amp; H) 5 cocci and each cocci containing four or more seeds (Figure 2A),</td>
<td>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 &amp; P) Fruit 2 seeded (Figure 2. J)</td>
</tr>
<tr>
<td><strong>Powder characters</strong></td>
<td>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)</td>
<td>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).</td>
</tr>
</tbody>
</table>
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).
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 2: Total phenolic and flavonoid content of fruit powder of *T. terrestris* and *P. murex*](image)

<table>
<thead>
<tr>
<th>Total phenolic and flavonoids</th>
<th><em>T. terrestris</em></th>
<th><em>P. murex</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content</td>
<td>28 mg</td>
<td>31 mg</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>0.785 mg</td>
<td>0.843 mg</td>
</tr>
</tbody>
</table>

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. Rᵢ 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 observed at Rᵢ 0.64 under UV254 nm, at Rᵢ 0.31 (corresponding to marker compound umbelliferon), Rᵢ 0.61 and Rᵢ 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).

![Table 3: Comparison of physico-chemical parameters of fruits of *T. terrestris* and *P. murex*](image)

<table>
<thead>
<tr>
<th>Physico-chemical parameters</th>
<th>Values %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T. terrestris</em></td>
</tr>
<tr>
<td>Moisture content</td>
<td>10.54</td>
</tr>
<tr>
<td>Water soluble extractive (w/v)</td>
<td>14.85</td>
</tr>
<tr>
<td>Alcohol soluble extractive (w/v)</td>
<td>4.98</td>
</tr>
<tr>
<td>Total ash (w/w)</td>
<td>3.28</td>
</tr>
<tr>
<td>Acid insoluble ash (w/w)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

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]. Based on the pharmacognotical, 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 asamri (breaking calculus) in classical texts. Hence the present study justifies the use of *P. murex* fruits as a substitute of *T. terrestris*.

5. Acknowledgments

Authors are thankful to TATA Trusts, Mumbai for the financial assistance and Arya Vaidya Sala, Kottakkal for extending facilities for conducting the study. Authors are also thankful to Dr. Indira Balachandran, Project Director, and Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal for extending support and encouragement for the study.

6. References


