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Physicochemical and Phytochemical Evaluation of Whole Plant, Fruit and Root of Gokshura (Tribulus terrestris Linn.)

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Abstract: *Gokshura (Tribulus terrestris Linn., Zygophyllaceae) is a classical Ayurvedic drug used mainly for urinary and reproductive complaints. Pharmacopoeial standards exist for its fruit and root, but none for the whole plant. We examined all three in samples collected in January 2026. Foreign matter, moisture, total and acid-insoluble ash, extractive values and pH were measured by Ayurvedic Pharmacopoeia of India methods. Methanolic, aqueous and decoction extracts were screened for major metabolite classes, ethanolic extracts were run on TLC, and methanolic extracts were profiled by HPLC. Root and fruit met every pharmacopoeial limit. Glycosides, steroids, terpenoids, saponins and flavonoids were detected, whereas alkaloids were not detected qualitatively. Saponin content was highest in the whole plant (1.87%) and flavonoid content in the fruit (1.62%). The results give working reference values for quality control.*

Keywords: Gokshura, *Tribulus terrestris*, physicochemical analysis, phytochemical screening, TLC, HPLC, Dravyaguna, quality control

I. INTRODUCTION

Gokshura is the Ayurvedic name for *Tribulus terrestris* Linn. (family Zygophyllaceae), a prostrate annual that grows as a common weed across the warm, dry parts of India [1,2]. The name joins *go* (cow) and *kshura* (hoof), because the mature fruit carries sharp spines that can pierce the hoof of a grazing animal. The classical texts rank it among the leading remedies for *mutrakrichhra* (dysuria) and *vata* disorders. Charaka counts it among the *mutrala* and *shothahara* herbs, Sushruta places it in the Vidarigandhadi *gana* and the Laghupanchamula, and Vagbhata lists it in a group indicated for *ashmari* (urinary calculi), *mutrakrichhra* and *shula* [3–5]. Its recorded properties are *madhura rasa*, *guru* and *snigdha guna*, *sheeta virya* and *madhura vipaka*, with a *vata–pitta* pacifying action [6].

Botanical identity alone does not guarantee the quality of a crude drug. Charaka's guidance on judging a drug, which covers its appearance, properties, place and season of collection, storage, preparation and dose, is in effect an early standardization scheme [7]; its modern counterpart is a set of pharmacopoeial tests for purity, extractable matter and chemical content [8,9]. The Ayurvedic Pharmacopoeia of India (API) gives limits for Gokshura fruit and root separately. The plant is, however, also used whole (*panchanga*) in decoctions and formulations, and we found no recorded limits for it [8,10]. The amount of active constituents, steroidal saponins in particular, varies with plant part, soil and climate [11], so values for one part cannot simply be assumed for another.

This study therefore compares the physicochemical and phytochemical profile of the whole plant, fruit and root of Gokshura drawn from a single collection, with the aim of providing reference data for each part.

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II. METHODOLOGY

2.1 Plant material

Whole plants, roots and fruits of *Tribulus terrestris* Linn. were collected from the field in January 2026. The material was identified and authenticated by a certified institute. Each part was powdered and passed through a No. 85 mesh sieve before analysis.

2.2 Physicochemical analysis

All tests followed the API procedures [8,12]. For foreign matter, 250 g of each sample was spread in a thin layer, inspected with the naked eye and a 6× lens, and the impurities were separated and weighed. Moisture was determined by drying 10 g of powder in a hot-air oven at 105 °C for 1½ h, cooling in a desiccator and reweighing. For total ash, 2 g of powder in a pre-weighed silica crucible was ignited in a muffle furnace at 450 ± 5 °C until free of carbon (about 6 h), cooled in a desiccator and weighed to constant weight. For acid-insoluble ash, the total ash was boiled with 25 mL of dilute hydrochloric acid and filtered through Whatman No. 41 paper, and the residue was ignited to constant weight. Extractive values were obtained by macerating 5 g of coarse powder in 100 mL of ethanol or water for 24 h (frequent shaking for the first 6 h, then standing for 18 h), filtering, evaporating 25 mL of filtrate to dryness at 105 °C and calculating the residue against the air-dried drug. For pH, 1 g of powder was stirred into 100 mL of water, left for 4 h and read on a digital pH meter calibrated with pH 4.0 and 7.0 buffers. Each parameter was determined separately for root, fruit and whole plant.

2.3 Preliminary phytochemical screening

Five grams of each powder was shaken with 100 mL of methanol on a rotary shaker for 6 h, left to stand for a further 18 h and filtered through Whatman No. 1 paper. Aqueous extracts and decoctions (*kwatha*) of the same three samples were screened in parallel. The tests used were Dragendorff's, Mayer's and Wagner's tests (alkaloids), a modified Borntrager test (glycosides), the Shinoda test (flavonoids), the froth test (saponins), ferric chloride (tannins), Liebermann–Burchard (steroids), tin–thionyl chloride (triterpenoids), Molisch's test (carbohydrates), iodine (starch) and Millon's reagent (proteins) [12,13]. Results were graded as absent (–) or present in increasing intensity (+, ++, +++).

2.4 Thin layer chromatography

Ethanolic extracts were applied with a capillary to silica gel 60 F254 aluminium plates and developed over 8 cm in toluene : ethyl acetate (8:1) after 20 min of chamber saturation, at room temperature (about 32 °C). Plates were examined under UV 254 nm and 366 nm, sprayed with vanillin–sulphuric acid, heated for 30 s and examined again. Rf was calculated as the distance travelled by the compound divided by the distance travelled by the solvent front.

2.5 High performance liquid chromatography

One gram of powder in 10 mL of methanol was refluxed on a water bath for 15 min, cooled and filtered through Whatman No. 1 paper, and the filtrate was passed through a 0.22 µm syringe filter. Analysis used a Shimadzu UFLC modular system with a C18 column at 27 °C, a gradient of 0.01% phosphoric acid in water and acetonitrile at 1.0 mL/min, 20 µL injections, detection at 254 nm and a 30 min run.

2.6 Quantitative estimation

Total steroids, total saponins, flavonoids and alkaloids were estimated in each sample and expressed as per cent of the drug.

III. LITERATURE REVIEW

Chemical work on *T. terrestris* has centred on steroidal saponins. The fruit is reported to contain terrestrosins, diosgenin, hecogenin, neotigogenin and related spirostanol glycosides, together with amides such as terrestribisamide

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and N-*p*-coumaroyltyramine, ferulic and *p*-hydroxybenzoic acids and β-sitosterol. The root is reported to contain diosgenin, neotigogenin, tigogenin, hecogenin and gitogenin, with campesterol, stigmasterol and β-sitosterol [10]. The furostanol saponins protodioscin and protogracillin are generally treated as the characteristic markers of the drug, although their content differs widely between sources [11,14]. The β-carboline alkaloid harmine has also been reported [14].

Reported pharmacological actions include diuretic and anti-urolithiatic effects, dilation of coronary vessels by the saponin fraction, an influence on the hypothalamic–pituitary–gonadal axis, and activity of alcoholic and aqueous extracts against *Escherichia coli* [11,14]. Pharmacognostical descriptions are available for several organs, including the flower [15].

Most quality-control data, including the pharmacopoeial monographs, relate to a single part of the plant. Comparative data on root, fruit and whole plant, obtained on one batch with identical methods, appear limited. Such data would also help separate Gokshura from *Pedalium murex* Linn. (*brihat gokshura*), a related drug with which it is often compared.

IV. RESULTS AND DISCUSSION

4.1 Physicochemical parameters

Table 1. Physicochemical parameters of Gokshura root, fruit and whole plant

Parameter	Root	API limit (root)	Fruit	API limit (fruit)	Whole plant
Foreign matter (%)	1.00	NMT 2	0.50	NMT 1	Nil
Moisture content (%)	3.70	–	7.41	–	7.81
Total ash (%)	6.97	NMT 13	13.50	NMT 15	19.90
Acid-insoluble ash (%)	1.44	NMT 3	1.49	NMT 2	3.70
Alcohol-soluble extractive (%)	14.20	NLT 4	14.87	NLT 6	7.75
Water-soluble extractive (%)	13.92	NLT 10	14.78	NLT 10	22.05
pH (1% in water)	6.00	–	6.50	–	6.95

NMT, not more than; NLT, not less than; –, no limit stated. No API limits are available for the whole plant. pH was read at 32.1 °C (root), about 32 °C (fruit) and 29.5 °C (whole plant).

Root and fruit satisfied every API limit (Table 1). Foreign matter was 1.00% in the root, 0.50% in the fruit and nil in the whole plant, which fits material collected straight from the field and handled with care. Moisture ranged from 3.70% in the root to 7.81% in the whole plant. None of the samples is wet enough to encourage microbial growth, though fruit and whole plant hold roughly twice the moisture of the root and need correspondingly careful storage.

Total ash rose from 6.97% in the root to 13.50% in the fruit and 19.90% in the whole plant. The root and fruit values sit below their limits of 13% and 15%. The high figure for the whole plant is probably partly intrinsic, since sections of this plant showed cluster and rosette crystals of calcium oxalate in several tissues. Its acid-insoluble ash of 3.70%, however, is more than double that of root and fruit and suggests that some siliceous matter, such as soil lodged among the hairy aerial parts, may also contribute. Because no limit exists for the whole plant, the figures are best read as provisional reference values and not as pass or fail results.

Root and fruit gave alcohol-soluble extractives of 14.20% and 14.87% and water-soluble extractives of 13.92% and 14.78%, all above the minimum limits. The whole plant behaved differently: its water-soluble extractive (22.05%) was nearly three times its alcohol-soluble extractive (7.75%). This points to a larger share of highly polar matter in the pooled aerial and underground tissue, consistent with the strong carbohydrate and glycoside reactions of its methanolic

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extract (Section 4.2), and to a smaller proportion of alcohol-extractable material. The pH of the aqueous suspensions was 6.00, 6.50 and 6.95 for root, fruit and whole plant, that is, slightly acidic to almost neutral.

4.2 Phytochemical screening

Table 2. Preliminary phytochemical screening of Gokshura extracts

Constituent	Methanolic extract			Aqueous extract			Decoction		
	Root	Fruit	Whole	Root	Fruit	Whole	Root	Fruit	Whole
Alkaloids	–	–	–	–	–	–	–	–	–
Glycosides	+++	++	+++	+	+	+	–	–	–
Flavonoids	+	+	+	–	–	–	–	+	+
Steroids	++	+	+++	–	–	–	–	–	+
Tannins	–	–	–	–	–	+	–	–	–
Terpenoids	++	+	+++	–	–	–	–	–	–
Saponins	++	+	++	++	+	+	+	+	+
Carbohydrates	+++	++	+++	–	–	–	–	–	–
Starch	–	–	–	–	–	–	–	–	–
Proteins	++	+	++	–	–	–	–	–	–

– absent; + present; ++ moderate; +++ abundant.

Methanol gave the richest and most intense spectrum in all three parts (Table 2). Glycosides, flavonoids, steroids, terpenoids, saponins, carbohydrates and proteins were present, while alkaloids, tannins and starch were absent. The whole plant scored highest for glycosides, steroids, terpenoids and carbohydrates, as would be expected of a pooled sample carrying constituents from both aerial and underground tissue. The cold aqueous extract was much narrower: glycosides and saponins appeared in all three parts, and only the whole plant showed a trace of tannin.

The decoction did not simply fall between the two. Glycosides detected in the cold aqueous extract were absent after boiling, whereas flavonoids in the fruit and whole plant, and steroids in the whole plant, appeared only in the decoction. Heat may have improved the release of these compounds, while prolonged heating may have hydrolysed some glycosides, although this was not tested. Saponins were detected in every extract of every part. The consistent absence of alkaloids and tannins agrees with the view that the chemistry of the plant is dominated by steroidal saponins [10]. Overall, the pattern shows that the choice of solvent and method changes the apparent composition at least as much as the choice of plant part.

4.3 Thin layer chromatography

Table 3. TLC profile of ethanolic extracts (toluene : ethyl acetate, 8:1)

Sample	Spots	Rf value	Colour / appearance
Root	1	0.48	Yellow-green spot
Fruit	1	0.46	Yellow-green fluorescent spot
Whole plant	4	0.25, 0.56, 0.68, 0.78	Pinkish red, bright red, yellowish green, bright red

Root and fruit each gave one prominent spot, at Rf 0.48 and 0.46 (Table 3). The two values are close enough to suggest a shared dominant component, although co-chromatography would be needed to confirm this. The whole plant resolved into four spots (Rf 0.25, 0.56, 0.68, 0.78), a more varied profile that matches its richer qualitative screening. The mobile phase is fairly non-polar, so the spots that migrated are more likely to be low-polarity constituents such as

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sterols, triterpenoids, sapogenins or flavonoid aglycones than intact saponin glycosides, which would remain near the baseline. Spot identities were not established in this work.

4.4 Quantitative estimation

Table 4. Content of major phytoconstituent groups (% of drug)

Constituent group	Root	Fruit	Whole plant
Total steroids	1.45	2.21	1.14
Total saponins	1.77	1.26	1.87
Flavonoids	0.63	1.62	0.80
Alkaloids	0.29	0.57	0.39

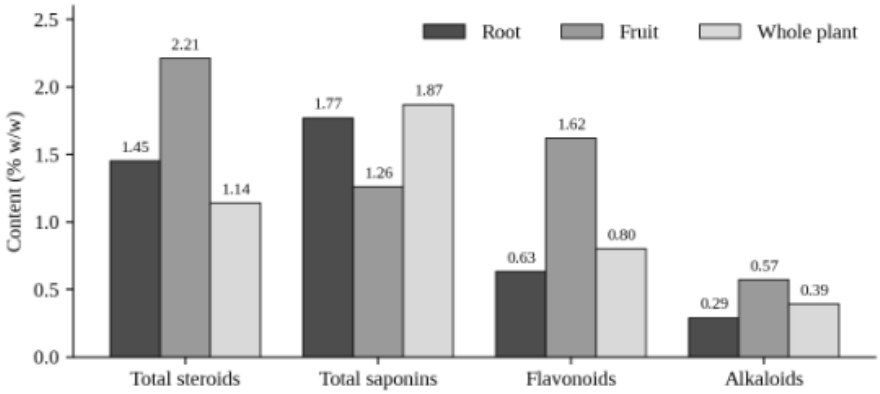


Figure 1. Phytoconstituent content of Gokshura root, fruit and whole plant

Total saponins were highest in the whole plant (1.87%) and root (1.77%) and lower in the fruit (1.26%), while the fruit led in total steroids (2.21%) and flavonoids (1.62%), the latter being more than twice the root value (Table 4, Figure 1). The Shinoda test gave only a single plus in all three methanolic extracts, so the qualitative grading was too coarse to show this difference. Alkaloids were negative in every qualitative test, yet 0.29–0.57% was recorded in the estimation. The estimate may include non-alkaloid nitrogenous compounds, or the alkaloids may be present below the level that gives a visible precipitate. Since harmine has been reported from this plant [14], the alkaloid status of this material deserves re-examination with a more specific method.

4.5 HPLC fingerprints

Table 5. Salient features of the HPLC chromatograms (254 nm)

Sample	Peak range (min)	Prominent peaks (retention time, min)	Profile
Root	3–5 (minor)	2.622 (about 72% of total area)	One dominant peak, few minor peaks
Fruit	3–20	8.421 (dominant); 3.225; 17.223; cluster at 13–19	Distributed multi-peak profile
Whole plant	3–28	14.44; 15.17; 17.28; 18.56	Most complex; broad spread of peaks

The three parts gave clearly different fingerprints (Table 5). The root chromatogram was dominated by one sharp peak at 2.622 min with a few minor peaks at 3–5 min. The fruit showed a spread of peaks over 3–20 min with its largest at

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8.421 min, and the whole plant was the most complex, with intense peaks between 13 and 19 min. These patterns fit the idea of a simpler, saponin-dominated root and a more mixed fruit and whole plant. The peaks are, however, only provisionally assigned. No reference compound was injected, the early root peak elutes close to the column dead time and may represent unretained polar material, and furostanol saponins such as protodioscin absorb weakly at 254 nm. The fingerprints are therefore useful for comparing and identifying batches but not yet for naming constituents. Repeating the analysis with protodioscin and diosgenin standards, at a lower detection wavelength or with ELSD or MS detection, would settle the question.

4.6 Overall comparison and limitations

Taken together, the root was the cleanest material, with the lowest ash and a saponin-rich, simple fingerprint. The fruit combined acceptable purity with the highest flavonoid and steroid contents. The whole plant was the most chemically varied but carried the highest ash, and its values await pharmacopoeial limits. The study has limits of its own: it rests on one collection, replicate statistics were not reported, and chromatographic peaks were not matched to standards. Further batches from different regions and seasons are needed before the figures can be adopted as standards.

V. CONCLUSION

Gokshura root and fruit from this collection met all API limits for foreign matter, ash and extractive values, and the whole plant gave a distinct set of values for which no limits yet exist. Methanol was the most effective solvent for revealing glycosides, steroids, terpenoids, saponins and flavonoids, and alkaloids were not detected qualitatively. Saponins were richest in the whole plant and root, and flavonoids and steroids in the fruit. TLC and HPLC fingerprints separated the three parts. These data can serve as preliminary reference values for identifying and quality-checking Gokshura, pending confirmation on more batches and with marker compounds.

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