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Comparative Phytochemical Profiling and In-Vitro α-Amylase Inhibitory Activity of Two Botanical Sources of Gojivha: Onosma Bracteatum Wall. and Elephantopus Scaber Linn.

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Abstract: ***Background: Gojivha is a botanically controversial Ayurvedic drug traditionally indicated in Prameha. Onosma bracteatum Wall. is the source recognised by the Ayurvedic Pharmacopoeia of India (API), whereas Elephantopus scaber Linn. is used as a regional substitute in Maharashtra and South India. Our aim is to test their comparative antidiabetic potential. Objective: To compare the pharmacognostic, phytochemical and in-vitro α-amylase inhibitory profiles of the two sources. Methods: Powdered whole plants were evaluated for organoleptic, microscopic and physicochemical parameters (API methods), preliminary and quantitative phytochemistry (total phenols, tannins, flavonoids), TLC and HPLC (gallic acid, quercetin). Ethanolic extracts (200–1000 µg/mL) were tested for α-amylase inhibition by the DNS method against acarbose, in triplicate. Data were analysed by two-way ANOVA with Tukey's HSD test. Results: O. bracteatum had higher total phenols (21.99% vs 16.92%), tannins (13.54% vs 7.03%), gallic acid (0.74% vs 0.15%) and quercetin (0.33% vs not detected); E. scaber had higher total flavonoids (0.63% vs 0.42%). Inhibition was concentration-dependent for all samples. At 1000 µg/mL, inhibition was 95.04% for acarbose, 74.78% for O. bracteatum and 71.37% for E. scaber. Sample, concentration and their interaction were significant (P < 0.001). Averaged over all concentrations, E. scaber (58.60%) was slightly more active than O. bracteatum (56.31%; mean difference 2.29%, 95% CI 0.45–4.14, P = 0.012), but the ranking reversed at 600 and 1000 µg/mL. Conclusion: Both sources inhibit α-amylase in a dose-dependent manner, with modest and concentration-dependent differences between them, and neither matched acarbose. The findings give preliminary support to E. scaber as a regional alternative source of Gojivha; fractionation, kinetic and in-vivo studies are needed.***

Keywords: *Gojivha; Onosma bracteatum; Elephantopus scaber*; α-amylase inhibition; *Prameha*

1. Introduction

Accurate identification of source plants is central to the safe and effective use of Ayurvedic drugs, yet a single classical name is often applied to more than one botanical species. *Gojivha* (Gaozaban, "cow's tongue") is a long-recognised example.

Classical Samhita and *Nighantu* texts describe it as a *laghu, tikta-kashaya, sheeta-virya*, *kapha-pitta-hara* drug used mainly externally and in polyherbal decoctions for fever, cough, wounds, skin disease, poisoning and *Prameha* [1].

Two botanical sources are in use. *Onosma bracteatum Wall.* (Boraginaceae) is the source recognised by the Ayurvedic Pharmacopoeia of India (API) [2]. It grows in the high-altitude western and central Himalaya and is not cultivated in Maharashtra [3]. *Elephantopus scaber Linn.* (Asteraceae) is abundant across Maharashtra and southern India and is used regionally as Gojivha [4]. Whether this accessible substitute is comparable to the official source has not been established.

Diabetes mellitus makes the question clinically relevant. The International Diabetes Federation estimated 589 million adults with diabetes worldwide in 2024, projected to reach 853 million by 2050, with 89.8 million in India [5]. Postprandial hyperglycaemia can be reduced by inhibiting pancreatic α-amylase, which hydrolyses dietary starch. Synthetic inhibitors such as acarbose are effective but frequently cause bloating, flatulence and abdominal discomfort, which limits adherence [6,7]. Plant-derived α-amylase inhibitors are therefore of continuing interest [8].

Since *Gojivha* is indicated in *Prameha* in the Nighantu literature, this study compares the two sources on three levels: pharmacognostic and physicochemical identity, phytochemical profile (including HPLC quantification of gallic acid and quercetin), and in-vitro α-amylase inhibitory activity against acarbose.

2. Materials and Methods

2.1 Plant material and authentication

O. bracteatum was obtained as raw drug from a local vendor in Himachal Pradesh (collected in October). *E. scaber* was collected fresh near Mannadi, Nagercoil, Tamil Nadu (December), washed, shade-dried and powdered (sieve no. 85). Both samples were authenticated by certified institutes (certificates held by the authors). Powders were stored in airtight plastic containers. Acarbose (standard pharmacy) was the positive control. Reagents included potato starch, 3,5-dinitrosalicylic acid (DNSA), Folin–Ciocalteu reagent and α-amylase.

2.2 Pharmacognostic and physicochemical evaluation

Organoleptic, macroscopic and microscopic (transverse section and powder) characters were recorded. Foreign matter, pH (1 g in 100 mL water, 4 h, digital pH meter calibrated at pH 4.0 and 7.0), total ash (2 g, 450 ± 5 °C), acid-insoluble ash, alcohol-soluble extractive (24 h cold maceration) and loss on drying (105 °C) were determined as per the API, each in triplicate.

2.3 Phytochemical analysis

Extracts in ethanol, methanol, hydroalcohol, petroleum ether and chloroform were screened by standard qualitative tests (Dragendorff, Shinoda, ferric chloride, froth, Molisch, Liebermann–Burchard and related tests) [13,14]. Ethanol gave the highest yield and was used for all further work. Total phenols, tannins and flavonoids were estimated quantitatively.

TLC was run on precoated silica gel 60 F254 plates (10 µL bands, 8 cm run). The mobile phase was n-butanol:acetic acid:water (4:1:5) for *O. bracteatum* (API) and toluene:ethyl acetate:formic acid (6:5:1) for *E. scaber*. Plates were examined in visible light, UV 254 nm and UV 365 nm and after iodine vapour.

HPLC used a Dionex Ultimate 3000 system with an Agilent Eclipse Plus C18 column (150 × 4.6 mm, 5 µm), isocratic elution for 10 min.

Parameter	Gallic acid	Quercetin
Mobile phase	o-phosphoric acid in water (pH 2.5): acetonitrile, 90:10	0.1% o-phosphoric acid: methanol, 50:50
Column temperature	40 °C	35 °C
Injection volume	10 µL	20 µL
Detection	272 nm	370 nm
Calibration range	0.5–25 ppm	2–100 ppm

2.4 α-Amylase inhibition assay

Inhibition was measured by the DNS (Bernfeld) method [15,16]. Ethanolic extracts and acarbose were tested at 200, 400, 600, 800 and 1000 µg/mL, each concentration in triplicate. α-Amylase solution (0.5 mg/mL, 500 µL) was pre-incubated with 500 µL of sample at 37 °C for 10 min. Then 500 µL of 1% starch was added and incubated at 37 °C for 10 min. The reaction was stopped with 1000 µL DNSA, and the tubes were heated at 85–90 °C for 5 min, cooled and diluted with 5 mL distilled water. Absorbance was read at 540 nm. An enzyme-only tube served as the control.

$$\text{Percentage Inhibition (\%)} = \left(\frac{\text{Control}-\text{Sample}}{\text{Control}}\right) \times 100$$

2.5 Statistical analysis

Values are mean ± SD (n = 3). Normality was checked by the Shapiro–Wilk test and homogeneity of variance by Levene's test. Effects of sample, concentration and their interaction were tested by two-way ANOVA, followed by Tukey's HSD test for pairwise comparison of samples. $P < 0.05$ was taken as significant.

3. Results

3.1 Pharmacognostic and physicochemical identity

Both powders were bitter (*tikta*) and rough (*ruksha*). *O. bracteatum* was light brown with no distinctive odour; *E. scaber* was brown with a herbaceous odour. Macroscopically *O. bracteatum* is a coarse, hairy shrub (about 40 cm) with a woody purplish-red root and deep blue trumpet-shaped flowers, whereas *E. scaber* is a smaller herb with a fibrous root and small mauve flower heads.



Image 1: Dried panchanga of *O.bracteatum Wall*



Image 2: Collected panchanga of *elephantopus scaber Linn.*

Three microscopic features separated the powders: trichomes (long, uniseriate, multicellular in O. bracteatum; unicellular, tapering and non-glandular in E. scaber), fibre form (striated and thick-walled vs slender and pointed), and calcium oxalate crystals (present only in O. bracteatum).

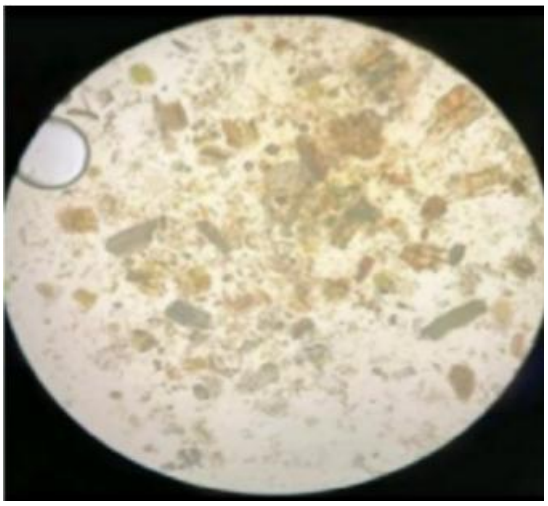


Image 3: Powder microscopy of *O.bracteatum*

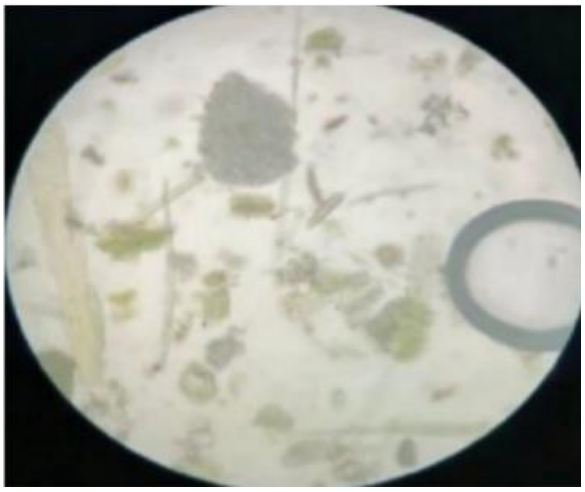


Image 4: Powder microscopy of *elephantopus scaber*

In transverse section, E. scaber had glandular and non-glandular trichomes and closed collateral bundles, against an open V-shaped arrangement in O. bracteatum.

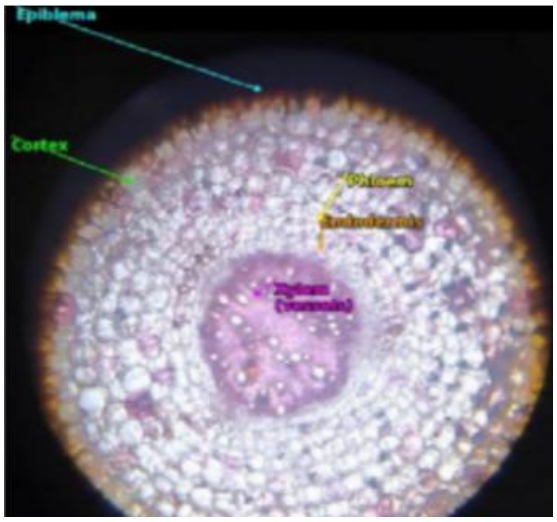


Image 5: T. S of Root of E.scaber

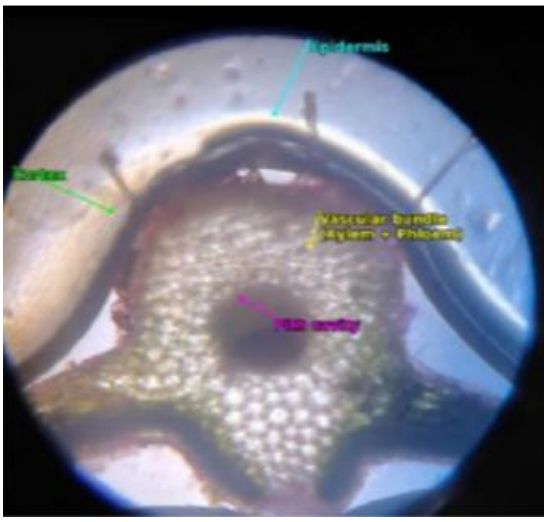


Image 6: T. S of leaf of E.scaber

No foreign matter was found in either sample, and acid-insoluble ash was about 3.5% in both. The two drugs differed in ash, extractive value and pH (Table 1). *O. bracteatum* met the API limits for total ash (not more than 26%) and acid-insoluble ash (not more than 4%).

Table 1: Physicochemical parameters

Parameter	O. bracteatum	E. scaber
Moisture content (%)	8.96	8.23
Total ash (%)	19.9	9.5
Acid-insoluble ash (%)	3.49	3.5
Alcohol-soluble extractive (%)	5.76	9.68
pH (1% aqueous)	8.49	6.07

3.2 Phytochemical profile

Ethanolic extracts of both plants contained alkaloids, phenols and tannins, terpenoids, flavonoids and saponins. Carbohydrates, glycosides and proteins were absent in both. Quantitatively, *O. bracteatum* was richer in phenols and tannins, and *E. scaber* in total flavonoids (Table 2). By HPLC, gallic acid was five times higher in *O. bracteatum*, and quercetin was detected only in *O. bracteatum.*

TLC resolved 7, 8 and 10 spots for *O. bracteatum* under visible light, UV 254 nm and UV 365 nm, and 6, 8 and 7 spots for *E. scaber*, giving a distinct fingerprint for each (Rf values in Supplementary Table S1).

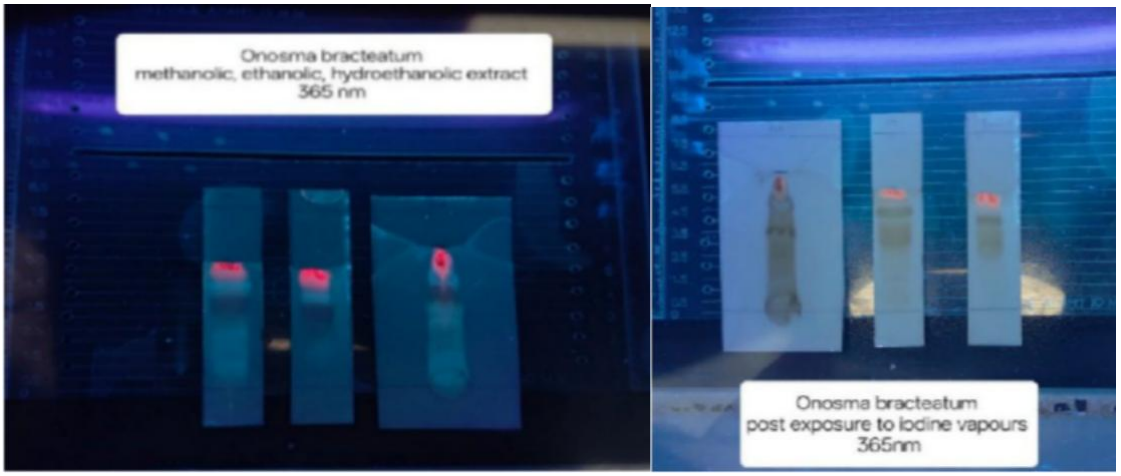


Image no. 7 and 8: TLC of Onosma bracteatum Wall

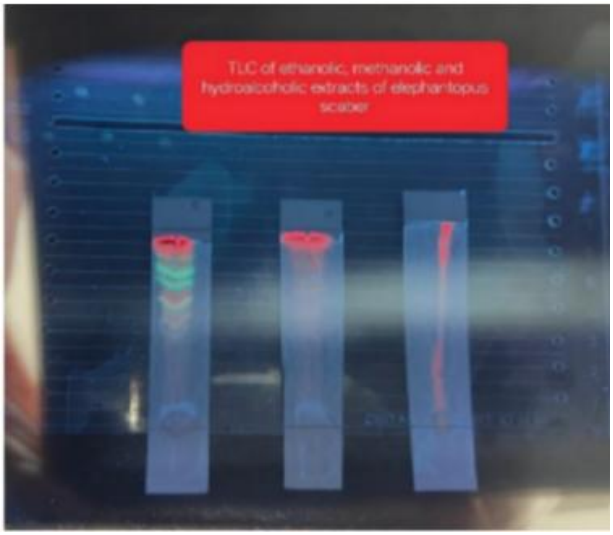


Image 9: TLC of Elephantopus scaber Linn

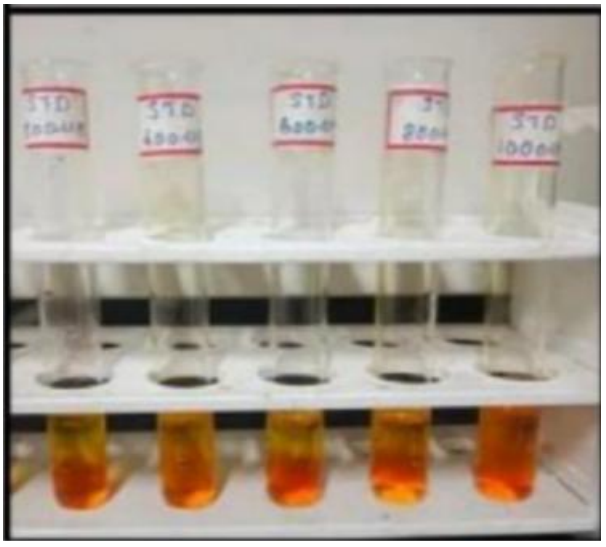


Image 10: Standard drug – Acarbose

Table 2: Quantitative phytochemical content

Constituent	O. bracteatum	E. scaber
Total phenols (%)	21.988	16.924
Total tannins (%)	13.541	7.028
Total flavonoids (%)	0.420	0.628
Gallic acid, HPLC (%)	0.74	0.15
Quercetin, HPLC (%)	0.33	Not detected

3.3 α-Amylase inhibitory activity

All three samples inhibited α-amylase in a concentration-dependent manner (Table 3). Acarbose was the most active at every concentration (64.85% at 200 µg/mL to 95.04% at 1000 µg/mL). *E. scaber* was more active than *O. bracteatum* at 200, 400 and 800 µg/mL, but *O. bracteatum* was more active at 600 and 1000 µg/mL.

Table 3: α-Amylase inhibition (% inhibition, mean ± SD, n = 3)

Concentration (µg/mL)	Acarbose	*E. scaber*	*O. bracteatum*
200	64.85 ± 2.54	44.25 ± 1.86	38.90 ± 2.25
400	69.09 ± 0.48	51.84 ± 4.58	46.54 ± 1.61
600	74.04 ± 2.41	57.61 ± 1.69	59.53 ± 1.00
800	81.66 ± 1.58	67.94 ± 0.79	61.79 ± 2.68
1000	95.04 ± 0.63	71.37 ± 0.80	74.78 ± 1.74
Overall mean	76.93	58.60	56.31



Image 11: Onosma bracteatum in increasing orders of concentration



Image 12: Elephantopus scaber in increasing orders of concentration Alpha amylase inhibitory activity assay – Images 10, 11, 12

Statistical analysis

The data were normally distributed in every group (Shapiro–Wilk, all $P > 0.05$), and variances were homogeneous (Levene $F = 0.483$, $P = 0.620$). Two-way ANOVA showed significant effects of sample ($F = 458.29$), concentration ($F = 318.85$) and their interaction ($F = 6.37$), each with $P < 0.001$ (df = 2, 4 and 8; error df = 30; $R^2 = 0.987$). The significant interaction means the dose response differed between samples, which fits the crossover seen at 600 and 1000 µg/mL.

Tukey's HSD test found all three samples different from one another (Table 4). Acarbose was about 18–21 percentage points above the extracts. *E. scaber* exceeded *O. bracteatum* by 2.29 percentage points on average.

Table 4: Tukey's HSD pairwise comparisons (mean difference in % inhibition)

Comparison	Mean difference	95% CI	P
Acarbose vs E. scaber	18.33	16.49 to 20.17	< 0.001
Acarbose vs O. bracteatum	20.63	18.79 to 22.47	< 0.001
E. scaber vs O. bracteatum	2.29	0.45 to 4.14	0.012

4. Discussion

The two sources of *Gojivha* differ clearly in identity and chemistry but inhibit α-amylase to a similar, modest degree. *E. scaber* was slightly more active overall (2.29 percentage points), yet *O. bracteatum* was more active at 600 and 1000 µg/mL. Because the interaction term was significant, the ranking depends on concentration and the pooled difference should not be read as a fixed advantage for either plant.

Identity and quality: Trichome type, fibre morphology and calcium oxalate crystals separate the two powders reliably, which supports their use for authentication. The API limits for total ash and acid-insoluble ash were met by *O. bracteatum*, and the acid-insoluble ash of E. scaber was similar. The higher alcohol-soluble extractive of *E. scaber* (9.68% vs 5.76%) fits ethanol as the extraction solvent for both. The pH difference (8.49 vs 6.07) is consistent with different inorganic and organic acid or base constituents; it was not investigated further here.

Chemistry versus activity: *O. bracteatum* contained more phenols, tannins, gallic acid and quercetin, which would be expected to favour enzyme inhibition, since phenolics and tannins bind and precipitate proteins including digestive enzymes [9–11]. *E. scaber* was nevertheless equal or better at most concentrations. Total phenol or flavonoid content therefore did not predict α-amylase inhibition in these crude extracts. Activity probably depends on which compounds are present and on additive or synergistic effects. *E. scaber* has a higher total flavonoid value but no detectable quercetin, so its flavonoids are likely other compounds; its sesquiterpene lactones and triterpenoids [4] may also contribute. These possibilities remain untested.

Comparison with acarbose: Neither extract approached acarbose (about 18–21 points lower on average). This is expected for crude, unfractionated extracts compared with a purified drug. At 1000 µg/mL the extracts reached 71–75%, which is a useful level of activity for crude material. Reported acarbose inhibition itself varies widely with assay conditions [8,12], so comparisons between laboratories should be made cautiously.

Implications for Ayurveda: *Gojivha* is indicated in *Prameha*, and both sources showed in-vitro activity relevant to that indication. The results give preliminary support to *E. scaber* as a regionally available alternative where the API source is regional. They do not establish therapeutic equivalence, because classical use is mainly external, the assay measures one enzyme in vitro, and the extract is not the traditional dosage form.

Limitations: Each concentration was tested in triplicate from a single sample of each plant, collected in different seasons and regions. No enzyme kinetics or cytotoxicity data were generated. Only α-amylase is reported here. The standard drug showed higher variability at 200 µg/mL than at other doses (SD 2.54). Results should be confirmed with multiple batches and geographic sources.

Future work: Priorities are bioactivity-guided fractionation with LC-MS to identify the active constituents of *E. scaber,* enzyme kinetic studies of the mode of inhibition, extraction-solvent optimisation, in-vivo testing and, later, clinical evaluation.

5. Conclusion

O. bracteatum and *E. scaber*, the two botanical sources of *Gojivha*, are distinguishable by simple pharmacognostic characters and differ in phenolic, tannin, flavonoid, gallic acid and quercetin content. Both inhibited α-amylase in a concentration-dependent way (71–75% at 1000 µg/mL), but less than acarbose (95%). *E. scaber* was marginally more active overall (2.29 percentage points, $P = 0.012$) while *O. bracteatum* was more active at the two highest doses, so the sources are broadly comparable. This gives preliminary support to *E. scaber* as a regional alternative source of *Gojivha*. Fractionation, kinetic and in-vivo studies are needed before substitution can be recommended.

Supplementary Table S1. TLC Rf values of ethanolic extracts

Detection	O. bracteatum	E. scaber
Visible light	0.17, 0.35, 0.42, 0.50, 0.56, 0.77, 0.81	0.51, 0.58, 0.66, 0.83, 0.85, 0.91
UV 254 nm	0.17, 0.29, 0.36, 0.48, 0.59, 0.70, 0.76, 0.82	0.18, 0.30, 0.51, 0.58, 0.66, 0.83, 0.87, 0.90
UV 365 nm	0.18, 0.28, 0.35, 0.48, 0.52, 0.56, 0.61, 0.70, 0.75, 0.85	0.27, 0.50, 0.58, 0.62, 0.83, 0.87, 0.90
Iodine vapour	0.17, 0.29, 0.36, 0.48, 0.59, 0.70, 0.76, 0.82	0.18, 0.30, 0.51, 0.58, 0.66, 0.83, 0.87, 0.90

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