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Nutritional and proximate evaluation of tubers from two underexplored endemic *Brachystelma* species of Northern Kerala, India

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Brachystelma ariyittaparensis and *B. vartakii* are underexplored endemic tuberous species of northern Kerala, India, with limited nutritional information. This study provides a comparative baseline evaluation of their proximate composition and vitamin profiles. Tubers were collected from naturally occurring populations in Kasaragod District and analysed using standard proximate methods and laboratory-validated vitamin procedures. The two species showed distinct proximate and vitamin profiles, with *B. ariyittaparensis* showing relatively higher protein and several vitamins, whereas *B. vartakii* showed relatively higher fat, fibre, and vitamins B1 and B2. These findings provide preliminary nutritional data for two poorly studied endemic taxa. However, mineral composition, antinutritional factors, bioavailability, toxicity, and safety require further investigation before wider dietary use can be recommended.

Keywords: *Brachystelma ariyittaparensis*, *Brachystelma vartakii*, Endemic species, Nutritional evaluation, Proximate composition, Underutilized tubers, Vitamin profile

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Introduction

The genus *Brachystelma* R.Br. comprises many species that remain poorly known and understudied in India, largely due to their short life span, ephemeral habit, and the presence of subterranean storage organs such as bulbs, corms, tubers, or rhizomes. Most species occur in arid and semi-arid regions of the country. Their short seasonal appearance and restricted distribution have resulted in their under-representation in early floristic accounts and herbarium collections.

Brachystelma R.Br. is the second largest genus of the tribe Ceropegieae within the subfamily Asclepiadoideae (Apocynaceae). The genus includes about 160 species distributed mainly in the Old World tropics. It occurs in sub-Saharan Africa, India, Sri Lanka, Southeast Asia, and northern Australia[1]. Following recent taxonomic revision, the genus comprises 38 taxa, of which 34 are native to India, including several newly described species and one hybrid[2]. Of these, 21 species are endemic to India. Several members of *Brachystelma* have been reported to possess notable medicinal and nutritional properties. The tubers of many species are consumed either raw or after processing by indigenous communities in Africa, Asia, and Australia[3-5]. Beyond their edibility, these plants also hold considerable ethnobotanical importance. In many regions, the tubers are utilised more extensively than other plant parts, highlighting their traditional value as a food resource.

Tubers of *Brachystelma* species are generally rich in moisture and nutrients, which enhances their potential as food sources. Owing to their high water content, nomadic communities and hunters in parts of East Africa have traditionally used them to quench thirst in water-scarce environments. Reports also indicate that wild animals such as hedgehogs, moles, and blesmols consume the ground tubers of *Brachystelma* species, which are considered non-toxic to both humans and animals[6]. Many species of the genus are additionally recognised for their therapeutic uses. Previous studies have documented the medicinal potential of species such as *B. edulis*, *B. naorojii,* and *B. togoense* in the treatment of ailments including stomach ache, headache, and wound healing[4,7,8].

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Among the numerous species of *Brachystelma*, nutritional information is currently available only for *B. edulis* and *B. naorojii*. Deshmukh and Rathod[4] reported that the tubers of *B. edulis* are comparatively rich in protein, fibre and carbohydrates when evaluated against common vegetables. Its carbohydrate content is comparable to that of African leafy vegetables such as gallwort, pigweed, and purslane. The fibre content of *B. edulis* (8%) is close to that reported for *Ceropegia hirsuta* (9.1%), and both exhibit similar protein levels. While leaves and tubers show comparable ash and moisture contents, the tubers possess higher dry matter (%DW). These findings support the nutritional potential of *Brachystelma* and indicate its possible value as a dietary supplement [4,9,10].

Despite increasing interest in wild edible and underutilised food plants in India, nutritional investigations remain concentrated on a limited number of species, while many endemic taxa are poorly characterised. Recent studies have emphasised the nutritional and functional importance of wild edible plants, indigenous foods, and lesser-known edible resources in India. *B. ariyittaparensis* and *B. vartakii* were selected because they are recently documented endemic tuberous species from the lateritic landscapes of northern Kerala, their tubers are reportedly consumed locally, and comparative nutritional information is unavailable. Therefore, the present study aimed to provide the first comparative baseline assessment of the proximate composition and vitamin profile of these two species, thereby addressing a specific knowledge gap and providing data relevant to future nutritional evaluation and conservation-oriented research[11-13].

Materials and Methods

Sample collection and preparation

Tubers of *Brachystelma ariyittaparensis* and *B. vartakii* were collected from naturally occurring populations on lateritic plateaus of Kasaragod District, Kerala, India, during June-August 2023. *B. ariyittaparensis* was sampled from four locations *and B. vartakii* from six locations. Mature tubers from 5-10 healthy individuals per location were randomly collected, depending on plant availability. The specimens were identified with the assistance of Dr P Biju, Associate Professor, Department of Botany, Government College, Kasaragod. Voucher specimens were prepared and deposited at the CALI Herbarium, University of Calicut, under accession numbers 7444 (*B. ariyittaparensis*) and 7443 (*B. vartakii*). The collected tubers were thoroughly washed with tap water, followed by distilled water, and sliced using a stainless-steel knife. The slices were shade-dried for two weeks to minimise degradation of heat-sensitive constituents.

The dried samples were powdered using a mortar and pestle, sieved, wrapped in aluminium foil, and stored in moisture- and air-resistant polyethylene bags at -18°C until analysis. For each species, material collected from individuals across the sampled locations was pooled to prepare a composite sample before analysis. All parameters described below were determined from the dried composite sample powder, except moisture content, which was measured immediately from fresh tubers by oven drying at 100°C to constant mass.

Determination of proximate composition

Proximate composition of the samples was determined using standard AOAC methods[14].

Moisture content (AOAC 925.10)

Exactly 5.000 g of fresh sample was weighed into a pre-weighed aluminium dish. The sample was dried in a hot-air oven at 100±2°C, cooled in a desiccator, and weighed. Drying, cooling, and weighing were repeated at 30-minute intervals until the difference between successive weights was less than 1 mg. Moisture content was calculated using:

$$Moisture\ (\%) = W1 - W/W1 - W2 \times 100$$

Where, W = weight of empty aluminium dish (g), W_1 = weight of dish + sample before drying (g), and W_2 = weight of dish + dried sample (g).

Crude protein content (AOAC 920.87)

Crude protein content was determined from total nitrogen using the Kjeldahl method. Exactly 1.000 g of dried sample was transferred to a Kjeldahl flask. Mercuric oxide (0.7 g) or copper sulphate, potassium sulphate (15 g), and concentrated sulphuric acid (40 mL) were added with glass beads to prevent bumping.

The mixture was digested gently until frothing ceased and then boiled steadily until a clear, pale-blue digest was obtained. After cooling, 200 mL of distilled water was added. The digest was made strongly alkaline by adding 110 mL of 450 g/L NaOH solution, followed by distillation.

The liberated ammonia was collected and titrated with 0.1 N NaOH using methyl red as the indicator. A blank determination was performed simultaneously.

$$Nitrogen\ (\%) = [(Blank - Titre) \times Normality \times 1.4] / Sample\ weight$$

$$Crude\ protein\ (\%) = Nitrogen\ (\%) \times 6.25$$

Crude fat content (AOAC 920.39)

2.5 g of dried sample was extracted with petroleum ether using a Soxhlet apparatus. Extraction was continued until exhaustive extraction was achieved. The solvent was evaporated, and the extraction flask was cooled in a desiccator and weighed to a constant mass. Crude fat (%) was calculated as:

$$Crude\ fat\ (\%) = 100\ (M1 - M2) / m$$

Where, M1 = mass of extraction flask with dried extract (g), M2 = mass of empty extraction flask (g), and m = mass of dried sample (g).

Crude fibre content (AOAC 962.09)

1 g of defatted sample was boiled in 1.25% sulphuric acid for 30 minutes, filtered, and thoroughly washed with hot distilled water. The residue was then boiled in 1.25% sodium hydroxide for 30 minutes, filtered, and washed again. The residue was dried at 105°C to constant weight and subsequently ashed in a muffle furnace at 550°C. Crude fibre content was calculated from the weight loss after ashing.

$$Crude\ fibre\ (\%) = [(W2 - W1) - (W3 - W1)] / Sample\ weight \times 100$$

Where, W_1 = Weight of the empty crucible or dish (g), W_2 = Weight of crucible + dried residue after acid and alkali digestion (before ashing) (g), and W_3 = Weight of crucible + ash after ignition in muffle furnace (g).

Determination of total carbohydrate and energy value

Total carbohydrate content was calculated by difference from the proximate composition according to the following equation:

$$Total\ carbohydrate\ (\%) = 100 - [Moisture\ (\%) + Ash\ (\%) + Crude\ protein\ (\%) + Crude\ fat\ (\%)]$$

Energy value was subsequently calculated from the protein, fat, and total carbohydrate contents using conventional energy conversion factors of 4 kcal/g for protein, 9 kcal/g for fat, and 4 kcal/g for carbohydrate, following FAO guidelines[15].

$$Energy\ (kcal/100\ g) = [Protein\ (g/100\ g) \times 4] + [Total\ fat\ (g/100\ g) \times 9] + [Total\ carbohydrate\ (g/100\ g) \times 4]$$

Dietary fibre content (AOAC 993.19 and 991.42)

Soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were determined using the Prosky enzymatic–gravimetric method. For SDF estimation, filtrates and washings were precipitated with 95% ethanol (400 mL), allowed to stand for 1 h, and filtered. The residue was washed sequentially with 78% ethanol, 95% ethanol, and acetone.

For IDF estimation, the residue was washed with water, 95% ethanol, and acetone. The residues were dried at 105°C for 5 h, cooled in a desiccator, and weighed (S_1 and S_2).

$$SDF\ (\%) = ((Residue\ S1 - Protein - Ash - Blank)/(Weight\ of\ test\ portion)) \times 100$$

$$IDF\ (\%) = ((Residue\ S2 - Protein - Ash - Blank)/(Weight\ of\ test\ portion)) \times 100$$

$$Total\ dietary\ fibre\ (\%) = SDF\ (\%) + IDF\ (\%)$$

Where, SDF: Soluble Dietary Fibre, IDF: Insoluble Dietary Fibre

Determination of vitamins

Vitamin analyses were performed using spectrophotometric, titrimetric, and chromatographic procedures.

Vitamin A (beta-carotene)

β-Carotene was determined spectrophotometrically using the water-saturated butanol extraction method[16]. Exactly 1.000 g of powdered sample was transferred to a 150 mL glass-stoppered Erlenmeyer flask, and 40 mL of water-saturated butanol (WSB; n-butanol: distilled water, 8:2, v/v) was added. The mixture was shaken vigorously for 1 minute and kept in the dark at room temperature for 16–18 h. It was shaken again and filtered through Whatman No. 1 filter paper. Absorbance of the clear filtrate was measured at 440 nm against WSB as a blank. A β-carotene stock standard (5 µg/mL) was prepared in WSB, and calibration standards of 0.25–1.50 µg/mL were used to construct the calibration curve. β-Carotene content was calculated from the calibration curve and expressed as µg/g (ppm).

Vitamin B1 (thiamine)

Vitamin B_1 (thiamine) was determined by the acid-dye spectrophotometric method based on formation of a thiamine–bromothymol blue ion-pair complex, followed by chloroform extraction[17]. A thiamine stock solution (1 mg/mL) was prepared in distilled water and diluted to 100 µg/mL. Aliquots (0.4–2.0 mL) of the working standard were transferred to 250 mL separating funnels, 2 mL of 0.2% bromothymol blue was added, and the aqueous volume was adjusted to 10 mL with distilled water. Chloroform (10 mL) was added, the mixture was shaken for 2 minutes, and allowed to separate. Absorbance of the chloroform layer was measured at 420 nm against the corresponding reagent blank. The sample was treated similarly, and thiamine content was determined from the calibration curve.

Vitamin B2 (riboflavin)

Vitamin B_2 (riboflavin) was determined by UV–Visible spectrophotometry based on established spectrophotometric approaches for riboflavin determination, with modifications[18,19]. Exactly 1.000 g of the sample was transferred to a 250 mL volumetric flask. Three millilitres of 1 M NaOH was added, and the flask was gently swirled, followed by 3 mL of glacial acetic acid; the volume was then made up to 250 mL with distilled water. A 25 mL aliquot was diluted to 100 mL, followed by a further 1:10 dilution. Riboflavin standards (2.5, 5.0, 7.5, and 10.0 mg/L) were prepared from a 100 mg/L stock solution using 1% acetic acid. The 5.0 mg/L standard and sample were scanned from 240 to 550 nm against 1% acetic acid as a blank, and the wavelength corresponding to the clearly defined maximum absorbance was selected for quantification. Riboflavin concentration was obtained from the calibration curve and corrected for the dilution factor.

Vitamin B3 (niacin)

Vitamin B_3 (niacin) was determined by UV–Visible spectrophotometry based on the method described by Chanda *et al.*, with modifications[20]. A niacin stock solution (100 µg/mL) was prepared in ethanol. A 2 µg/mL reference solution was scanned between 200 and 400 nm, and maximum absorbance was observed at 262 nm. For sample analysis, exactly 1.000 g of powdered sample was extracted with ethanol and made up to 100 mL. The solution was filtered through Whatman No. 41 filter paper and suitably diluted to 10 µg/mL. Absorbance was measured at 262 nm against ethanol as a blank, and niacin content was calculated from the calibration curve.

Vitamin C (ascorbic acid)

Vitamin C (ascorbic acid) was determined by iodometric titration following Satpathy *et al.*, with modifications[21]. Exactly 1.000 g of sample was extracted with 50 mL of distilled water in a 250 mL conical flask; approximately 100 mL of distilled water and 1 mL of starch indicator were then added. The sample was titrated with 0.005 mol/L iodine solution to the first permanent dark blue-black endpoint. Titrations were repeated until concordant titres (within 0.1 mL) were obtained.

The amount of ascorbic acid was calculated from the iodine consumed, using the equivalence: 1 mL of 0.005 mol/L iodine = 0.88 mg ascorbic acid, and expressed as mg/100 g of sample.

$$Vitamin\ C\ (mg/100\ g) = (V \times C \times 176.12 \times 100)/(sample\ mass)$$

Where V is iodine volume, and C is iodine molar concentration.

Vitamin D

Vitamin D was estimated by the antimony trichloride colourimetric method following chromatographic purification, based on De Witt and Sullivan, with modifications[22]. An accurately measured sample portion was saponified with alcoholic potassium hydroxide under reflux. The unsaponifiable fraction was extracted with solvent hexane, washed to neutrality, dried, and concentrated. The extract was purified successively on two chromatographic columns, and the purified residue was dissolved in ethylene dichloride. Ergocalciferol was used as the reference standard. Colour was developed with antimony trichloride reagent, and absorbance was measured at 500 nm, 45 seconds after reagent addition; correction readings were obtained at 550 nm as prescribed by the laboratory protocol. Vitamin D content was calculated by comparison with the ergocalciferol standard and expressed as µg/100 g.

Vitamin E

Vitamin E was determined spectrophotometrically using a modified colourimetric procedure for tocopherol estimation[23]. Exactly 1.000 g of powdered sample was mixed with 10 mL of ethanolic H_2SO_4 and refluxed for 30 minutes. After cooling, the

mixture was transferred to a separating funnel and extracted three times with 30 mL portions of diethyl ether. The combined ether extracts were evaporated at room temperature, and the residue was dissolved in 10 mL of absolute ethanol. Aliquots (1 mL) of the sample extract and vitamin E standard solution were taken separately in different test tubes. To each tube, 5 mL of absolute ethanol and 1 mL of concentrated nitric acid were added. The mixtures were allowed to stand for 5 minutes, and absorbance was measured at 410 nm. Vitamin E content was calculated using the laboratory calibration procedure and expressed as mg/100 g.

Statistical analysis

All analytical determinations were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). The triplicates represented analytical replicates of the composite samples rather than independent biological replicates. Accordingly, the data were used to describe analytical variability, and no inferential statistical comparison between the two species was performed.

Results and Discussion

Proximate composition

The proximate profiles of the two *Brachystelma* tubers are presented in Table 1 and Fig. 1. The two species showed different measured values for several proximate parameters. Ash represents total inorganic residue and should not be interpreted as evidence of specific mineral abundance in the absence of direct mineral analysis. Accordingly, the present data indicate differences in total ash only, and no claims regarding individual mineral elements are made.

B. ariyittaparensis showed higher measured moisture and protein values than *B. vartakii*, whereas *B. vartakii* showed higher fat, crude fibre, dietary fibre, total sugar and reducing sugar values. These interspecific differences may reflect species-specific

Table 1 — Proximate composition of *B. ariyittaparensis* (BA) and *B. vartakii* (BV) tubers

Parameters	*B. ariyittaparensis*	*B. vartakii*
Moisture (%)	1.83±0.03	0.23±0.01
Ash (%)	5.13±0.11	4.83±0.15
Fat (%)	3.47±0.12	4.63±0.15
Total carbohydrate (%)	81.34	82.94
Crude fibre (%)	2.26±0.25	2.80±0.10
Dietary fibre (%)	4.23±0.25	4.77±0.21
Energy (kcal/100 g)	389.40±0.36	402.90±1.15
Protein (%)	8.23±0.25	7.37±0.15
Total sugar (%)	1.53±0.15	2.17±0.15
Reducing sugar (%)	1.10±0.10	1.93±0.12

Values for measured parameters are expressed as mean ± standard deviation (n = 3 analytical determinations). Total carbohydrate was calculated by difference from the mean proximate values.

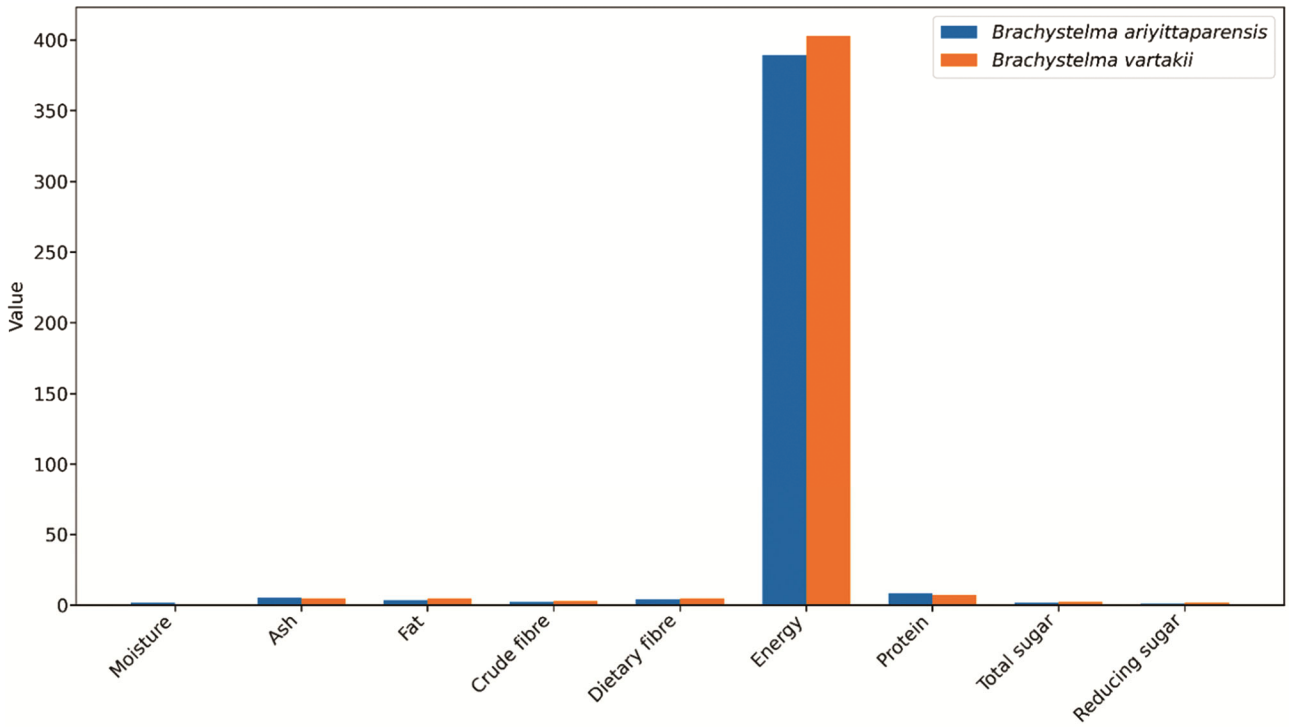

Fig. 1 — Comparative proximate composition of *B. ariyittaparensis* and *B. vartakii* tubers. Values are expressed as mean ± SD (n = 3 analytical determinations).

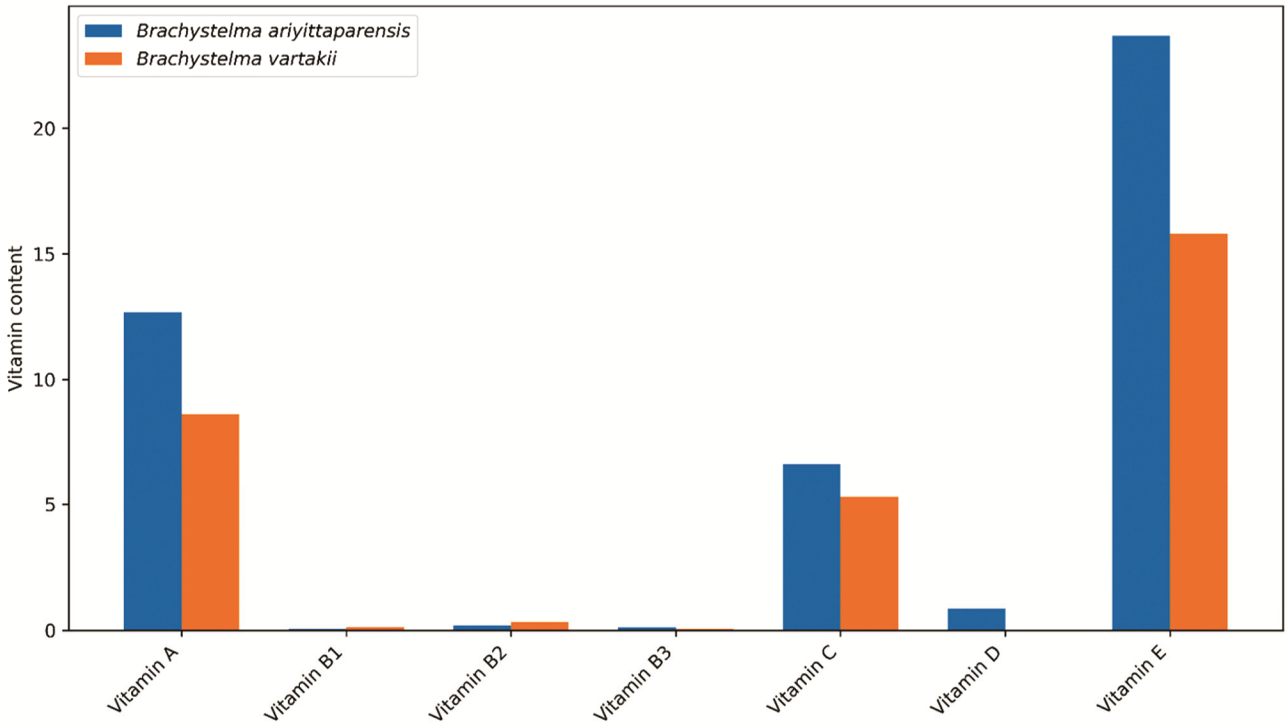

Fig. 2 — Comparative vitamin composition of *B. ariyittaparensis* and *B. vartakii* tubers. Vitamins A and D are expressed in µg/100 g; vitamins B1, B2, B3, C, and E are expressed in mg/100 g. Values are mean ± SD (n = 3 analytical determinations).

physiology, storage-reserve allocation, genotype, developmental stage, and environmental conditions at the collection sites; however, the present study was not designed to distinguish among these mechanisms. Variation in nutrient and antinutrient composition among tuber crops is well documented, and comparison across species should consider differences in plant material and analytical basis[24].

The observed fibre and macronutrient differences provide useful baseline compositional information. Dietary fibre is physiologically relevant and has been associated with favourable metabolic responses in controlled dietary studies; nevertheless, the present compositional data alone do not establish a health benefit from these *Brachystelma* tubers[25,26]. Antinutritional factors, mineral composition, digestibility, bioavailability, and safety were not assessed in this study and therefore limit the interpretation of the food value of these tubers.

Vitamins

Vitamin analysis showed distinct profiles in the two *Brachystelma* tubers (Table 2; Fig. 2). *B. ariyittaparensis* contained higher measured amounts of vitamins A (12.67±2.08 µg), B3 (0.11±0.01 mg), C (6.63±0.12 mg), D (0.87±0.09 µg), and E (23.67±1.53 mg), whereas *B. vartakii* contained higher amounts of vitamins B1 (0.13±0.01 mg) and B2 (0.32±0.02 mg).

Table 2 — Vitamin composition of *B. ariyittaparensis* and *B. vartakii* tubers (per 100 g dry sample, dry-weight basis)

Vitamin	*B. ariyittaparensis*	*B. vartakii*
Vitamin A (µg)	12.67±2.08	8.63±0.15
Vitamin B1 (mg)	0.05±0.01	0.13±0.01
Vitamin B2 (mg)	0.18±0.02	0.32±0.02
Vitamin B3 (mg)	0.11±0.01	0.06±0.01
Vitamin C (mg)	6.63±0.12	5.30±0.10
Vitamin D (µg)	0.87±0.09	< detection limit
Vitamin E (mg)	23.67±1.53	15.80±0.20

Values are expressed as mean ± standard deviation (n = 3 analytical determinations).

Because vitamins A and D are reported in micrograms while the other vitamins are reported in milligrams, these values should not be compared directly on a common numerical scale.

The vitamin data extend the limited compositional information available for *Brachystelma* and provide a basis for comparison with other edible tubers. Carotenoid accumulation in tubers can vary substantially with genotype and metabolic regulation, while vitamin occurrence and concentrations in plants are influenced by species, tissue, developmental, and environmental factors[27,28]. Accordingly, comparisons with cassava, yam, sweet potato, or other foods should be made cautiously because cultivar, dry/fresh weight basis, processing, maturity, and analytical method can

influence reported concentrations. The present results therefore support the interpretation of these species as underexplored food resources that require further study, rather than as established dietary sources of specific vitamins. Mineral composition, antinutritional factors, bioavailability, toxicity, and dietary exposure were not evaluated and should be addressed before making nutritional or food-use recommendations.

Conclusion

This study provides baseline comparative data on the proximate composition and vitamin profiles of the endemic tubers *B. ariyittaparensis* and *B. vartakii.* The species differed in several measured compositional parameters, indicating interspecific variation in storage reserves and vitamin profiles. These findings expand the limited nutritional information available for Indian *Brachystelma* species and support further investigation of these underutilised plants. The study is limited by its use of composite samples, its reliance on analytical rather than independent biological replication, and its restricted compositional scope. Mineral composition, antinutritional factors, phytochemicals, digestibility, bioavailability, and toxicity were not evaluated. Consequently, the present results should not be interpreted as evidence supporting unrestricted dietary or nutraceutical use. Further multidisciplinary studies, together with assessments of sustainable cultivation and conservation, are required before wider food use can be considered.

Conflict of interest

The authors declare that they have no conflict of interest.

AI use disclosure

Generative AI tools were used only for language editing and organisation of responses during manuscript revision. The authors reviewed and take full responsibility for the scientific content, data, analyses, and conclusions.

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