

Maesa bengalensis: Unlocking the hidden nutritional treasures and medicinal potential of an underutilized wild vegetable from Manipur, India

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This study assessed the nutritional composition, minerals, amino acids, anti-nutritional qualities, toxicity, and antioxidant activities of *Maesa bengalensis*, a wild edible plant from Manipur, India. Standard techniques were used for proximate analysis, minerals content, and anti-nutrient composition. HPLC analysis was conducted for vitamins, phenolics, and free amino acids using a Dionex Ultimate 3000 liquid chromatograph. The plant exhibited diverse mineral concentrations and a significant protein level (3.10±0.09%). Eighteen free amino acids were identified, with the highest amount of L-histidine (2.68 µg/mg) in the water extract and the lowest amount of L-methionine in the benzene extract. The aqueous extract showed substantial phenolic (36.08±1.92 mg/100 g) and flavonoid (19.94±2.35 mg/100 g) contents. Water-soluble B vitamins were present in varying amounts (0.24-18.49 mg/100 g), along with a high concentration of vitamin C (93.67±4.12 mg/100 g). The aqueous extract contained abundant phenolic compounds, such as syringic acid (18.01±0.33 µg/mg dry extract) and quercetin (37.56±0.53 µg/mg dry extract). The levels of antinutrients and heavy metals were below harmful thresholds, and the toxicity study confirmed the plant's safety for human consumption. These findings highlight the potential of *M. bengalensis* as a health food, nutraceutical, and dietary supplement, with prospects for development and commercialization in Manipur and neighbouring regions.

Keywords: Amino acid, Antinutritional, Antioxidant, *Maesa bengalensis*, Nutritional, Toxicity, Vitamins

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Wild edible plants (WEPs) are crucial to the diets and cultures of indigenous communities, serving as both staple and supplementary food sources. Rich in micronutrients, they are often used for treating various diseases. Many WEPs surpass common vegetables in nutritional value, with higher levels of vitamins, amino acids, phenolic acids, flavonoids, and strong antioxidant properties¹.

The northeastern region of India, including Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and Sikkim, is a biodiversity hotspot. Its diverse topography and climates support the growth of various wild species. India has around 800 WEP species, with 300 used by tribal communities in the northeast for food and medicine. These communities employ traditional methods to make these plants palatable. The region's diverse food offerings reflect its dietary diversity and adequate micronutrient intake^{2,3}.

During an ethnobotanical study of wild edible vegetables commonly used by the Mao Naga tribe of Manipur, *Maesa bengalensis* Mez. was identified as a frequently consumed species. It is known locally as “Kohra vu” in the Mao dialect, where “Kohra” translates to ‘clean up’ and “vu” means vegetable, this plant is traditionally recommended to individuals recovering from illness to help cleanse the body and promote faster recovery.

Maesa bengalensis is a shrub with smooth, glabrous, slender branches bearing few lenticels. Its simple, alternate leaves have 2-3.2 cm long, channeled petioles. The broadly ovate to sub-orbicular lamina (13×7.5-8.3 cm) may be rhomboid, with a cuneate base, deeply crenate margin, and attenuate apex. Both surfaces are glabrous, with distinct mid and lateral veins branching before the crenation teeth.

The inflorescence is axillary, racemose, 2-3 times branched, glabrous, and pedunculate. Peduncles and pedicels are slender and smooth. Flowers are actinomorphic, pentamerous, and bracteate; bracts are

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lanceolate with entire or rarely crenate margins and acute apices. Two lanceolate bracteoles (1-1.2 mm) have entire margins and acute apices. Resin rays are present. Sepals, five and gamosepalous (1 mm), have entire margins, broadly obtuse apices, and ventral resinous hairs extending to the sub-apex.

The five gamopetalous petals (2 mm) have entire margins and rounded, crisped apices. Petal rays form a basal bundle, branching dichotomously upward, fused halfway or slightly above. Stamens, inserted at the petal base, are exerted at maturity. Anthers (1 mm) are 2-celled, ovoid, dorsifixed, and dehisce longitudinally, with filiform filaments twice their length.

The ovary is half-superior and glabrous, with a slender style and faintly lobed stigma. The gynoecium is about 1 mm. Fruits are small berries (3 mm) with a persistent calyx covering half or more, and the style with stigma is visible on young fruits.

This is the first report on *M. bengalensis*, a species previously limited to Manipur and Meghalaya with scarce data. In India, 16 taxa of the *Maesa* genus are documented, with *Maesa indica* known for its edible fruits and *Maesa lanceolata* used in treating snake bites⁴. Motivated by traditional knowledge, this study scientifically evaluates *M. bengalensis* for its nutritional, anti-nutritional, antioxidant, and toxicity properties.

Materials and Methods

Plant materials

Maesa bengalensis plant materials were collected from Manipur, India, and its identification was authenticated at our office. A voucher specimen was preserved in the Plant Chemistry department under the registry number BSI/AAMAO/001. The leaves were carefully dried, ground into powder, stored in an airtight container, and used to assess the proximate composition, minerals, vitamins, amino acid, antioxidant properties, antinutritional factors, and toxicity through comprehensive studies.

Nutritional and mineral composition

The nutritional profile such as ash, moisture, fat, protein, and energy of the edible plant was determined using traditional food analysis techniques as outlined by the Association of Official Analytical Chemists⁵ and carbohydrate content was determined using the method provided by Hedge and Hofreiter⁶. The ash obtained from this plant, was digested in 5% hydrochloric acid (HCl) to prepare a suitable solution

for mineral element analysis using atomic absorption spectroscopy (AAS). Standard solutions for each element were prepared, and the estimation of minerals was performed^{5,7}.

Determination of amino acids

Total amount of free amino acids and the quantification of individual amino acids in the water, methanol and benzene extracts was analyzed using HPLC. The identification and quantification of amino acids was done using a photodiode array detector at four different wavelengths (260, 324, 338, 390 nm) based on the absorption maxima of the compounds under investigation⁸.

Water soluble vitamin by HPLC

The HPLC analysis was conducted to estimate water soluble vitamin in the investigated plant⁹. The mobile phase consisted of acetonitrile (Solvent A) and aqueous trifluoroacetic acid (TFA, 0.01% v/v) (Solvent B). Detection of the vitamins was performed using a photodiode array detector at four different wavelengths (210, 245, 275, 290 nm), corresponding to the absorption maxima and retention time of the vitamins.

Antioxidant properties

Preparation of plant extracts

Plant material (10 g) was extracted twice with water at room temperature, with stirring for 18-24 h each time. The resulting extracts were combined, and the solvent was concentrated and freeze dried to obtain crude extract. The same procedure was followed to prepare extracts using methanol and benzene as solvents. The dried extracts from each solvent were stored at -20°C.

The quantification of total phenolic, flavonoid, and flavonol content in the raw extracts was conducted using the procedure elucidated by Seal *et al.*¹⁰. Additionally, the extract's reducing power, free radical scavenging potential, metal chelating ability, and anti-lipid peroxidation efficacy were evaluated using the methodology detailed in the work by Seal *et al.*¹⁰.

Quantification of phenolic acids and flavonoids in the plant extracts was performed using HPLC analysis, following the method outlined by Datta *et al.*¹¹. The mobile phase utilized in the chromatographic separation comprised of methanol (Solvent A) and a 0.5% aqueous acetic acid solution (Solvent B), while the column temperature was

maintained at 25°C. Gradient elution was achieved by adjusting the ratio of Solvent A to Solvent B. The total analysis time for each sample was 105 min. HPLC chromatograms were detected at three different wavelengths (272, 280, 310 nm) using a photodiode array UV detector.

Anti-nutritional composition

Oxalate content in the plant material was estimated following the method described by Munro and Bassir, 1980¹². To determine the phytate content, Reddy and Love's method¹² was employed. The estimation of saponins was carried out following the procedure described by Hudson and El-Difrawi¹². Tannins were measured using vanillin-HCl technique by Price *et al.*, with tannic acid used as the reference standard¹². The cyanogenic glycoside content was assessed using the alkaline titration method, where the endpoint was determined by persistent turbidity against a black background⁵.

Toxicity study

Powdered plant materials (5 g) were macerated in 50 mL of distilled water at room temperature for 24 h. The mixture was then filtered to remove solid particles, and the filtrate was concentrated using a rotary evaporator under reduced pressure. The concentrated extracts were stored at -20°C until further use.

The haemolytic toxicity of the aqueous extract was assessed using the method described by Malagoli in (2007)¹². For the cytotoxicity study, fresh goat livers were obtained and hepatocytes were isolated using collagenase treatment. The isolated hepatocytes were incubated with varying concentrations (100-1000

µg/mL) of the aqueous extract and the cells were treated with MTT solution and incubated until purple intracellular formazan crystals formed. The formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and the absorbance of the solution was measured at 570 nm to evaluate hepatotoxicity¹².

The genotoxicity study was conducted using the single-cell gel electrophoresis comet analysis, as described by Singh *et al.* in (1988)¹². The DNA damage was evaluated by observing the dyed nuclei using fluorescence microscopy and determining the Olive Tail Moment (OTM) using comet assay software.

The Institutional Animal Ethics Committee (Approval No.-04/P/S/IAEC/2017), Serampore College, West Bengal, India, granted ethical permission for experiments on rats to obtain erythrocytes in accordance with CPCSEA norms. Fresh goat liver was obtained from a local butcher within 30 min of its death.

Statistical analysis

The data was analysed using triplicate samples, and the results were provided as the mean standard error mean (SEM). Statistical analysis was carried out by Tukeys test at 95% confidence level and statistical significance was accepted at the $p < 0.05$ level. SPSS software (version 11.0 for Windows) was used to conduct statistical analysis.

Results and Discussion

Nutritional composition

Table 1 provides an overview of the nutritional composition of *M. bengalensis* leaves. The data

Table 1 — Nutritional composition, minerals, vitamin and antinutritional content in *Maesa bengalensis*

| Nutritional composition | Amount | Minerals | Amount (mg/100 g) | Vitamin | Amount mg/100 g dry plant material |
|------------------------------------|-------------------|---------------------|----------------------|------------|------------------------------------|
| Ash (%) | 12.73±0.76 | Sodium (Na) | 1.54±0.24 | Vitamin C | 93.67±4.12 |
| Moisture (%) | 58.81±2.12 | Potassium (K) | 63.72±1.67 | Vitamin B1 | 3.74±0.16 |
| Protein (%) | 3.10±0.09 | Calcium (Ca) | 115.60±2.93 | Vitamin B2 | 0.24±0.02 |
| Fat (%) | 0.18±0.023 | Copper (Cu) | 0.19±0.04 | Vitamin B3 | 0.27±0.02 |
| Carbohydrate (%) | 6.42±0.028 | Zinc (Zn) | 7.84±0.62 | Vitamin B5 | 3.61±0.002 |
| Crude fibre (%) | 7.27±0.68 | Magnesium (Mg) | 28.84±1.08 | Vitamin B6 | 6.75±0.44 |
| Energy (kcal/100 g) | 39.76±1.12 | Iron (Fe) | 12.12±0.69 | Vitamin B9 | 18.49±1.52 |
| Antinutritional composition | Amount (%) | Manganese (Mn) | 1.45±0.19 | | |
| Oxalate | 0.24±0.08 | Heavy metals | Amount (µg/g) | | |
| Phytate | 0.21±0.03 | Lead (Pb) | 0.0263±0.0010 | | |
| Saponin | 0.16±0.09 | Cadmium (Cd) | BDL | | |
| Tannin | 0.17±0.03 | Arsenic (As) | 0.95±0.0007 | | |
| Cyanogen glycoside | 0.0052±0.0002 | Mercury (Hg) | 0.14±0.002 | | |

BDL: Below detection limit

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

displays protein content at $3.10 \pm 0.09\%$, crude fat at $0.18 \pm 0.023\%$, crude fiber at $7.27 \pm 0.68\%$, and carbohydrate content at $6.42 \pm 0.028\%$. Remarkably, the ash content is notably high, measuring at $12.73 \pm 0.76\%$, underscoring a significant mineral presence.

The protein content of $3.10 \pm 0.09\%$ in *M. bengalensis* is moderate compared to other commonly consumed vegetables. For instance, it is slightly higher than spinach, which has approximately 2.9% protein, and significantly more than low-protein vegetables such as cucumbers (0.6%), tomatoes (1.0%), and carrots (0.9%). On the lower end, potatoes contain about 2.0% protein, and lettuce provides 1.4%. However, *M. bengalensis* falls below certain high-protein vegetables like asparagus, which has 3.7% protein, and peas, which contain about 5.4%^{13,14}. This places *M. bengalensis* in the category of moderately good plant-based protein sources, offering more protein than many common vegetables while still being less rich than legumes like peas.

M. bengalensis contains 6.42% carbohydrates, which is lower than starchy vegetables like potatoes (17%) and sweet corn (19%), but comparable to non-starchy vegetables like broccoli (6.6%). Vegetables such as lettuce (2.5%) and spinach (2.9%) have less, while cabbage (4.6%) contains slightly fewer carbohydrates. In contrast, cauliflower (7.6%), broad beans (7.2%), and colocasia leaves (6.8%) have higher levels, with carrots at 10.6%¹⁴. Thus, *M. bengalensis* falls into the moderate carbohydrate range, offering more than leafy greens but less than starchy vegetables.

The fat content of *M. bengalensis* is notably low at 0.18%, making it similar to other low-fat vegetables. For example, carrots contain about 0.2% fat, and lettuce has around 0.3%. Vegetables like broad beans, cabbage, and potatoes have even lower fat contents, around 0.1%. On the higher end, cauliflower contains 1.3% fat, and curry leaves have 1.0%. In comparison, avocados, known for their high fat content, contain up to 15% fat¹⁴. The minimal fat content in *M. bengalensis* makes it ideal for low-fat diets, supporting obesity management and healthy eating habits aimed at reducing fat intake.

The significant crude fiber content of *M. bengalensis* ($7.27 \pm 0.68\%$) exceeds that of many commonly consumed fruits and vegetables, making it a valuable dietary component. For comparison, vegetables like spinach (2.2 g/100 g), broccoli

(2.6 g/100 g), and carrots (2.8 g/100 g) all contain much lower fiber levels than *M. bengalensis*. Even sweet potatoes, recognized for their fiber richness, offer 3.3 g/100 g, less than half the fiber content of *M. bengalensis*. Among wild vegetables, amaranth leaves, which are known for their high fiber content, contain 7.0 g/100 g, similar to this species. In contrast, other vegetables like cabbage (1.0 g/100 g), colocasia leaves (2.9 g/100 g), and broad bean leaves (3.7 g/100 g) have lower fiber content¹⁴.

However, *M. bengalensis* is outdone by a few plants, such as sweet potatoes (8.0 g/100 g), black beans (8.7 g/100 g), and fetid cassia (10.4 g/100 g). The high fiber content, especially insoluble fiber, in *M. bengalensis* promotes digestive health by adding bulk to stool, thereby facilitating effective food waste removal and maintaining regular bowel movements. This also helps prevent constipation and reduces the risk of gastrointestinal conditions like diverticulitis. Moreover, fiber helps lower cholesterol, regulate blood sugar levels, and reduce the risk of chronic diseases such as cardiovascular disease and type 2 diabetes. Therefore, the higher fiber content of *M. bengalensis*, compared to other vegetables, makes it highly effective for gut cleaning and overall digestive well-being. Incorporating this wild species into the diet can significantly aid in waste removal and long-term health^{14,15}.

Minerals content

Mineral analysis of *M. bengalensis* leaves reveals substantial levels of potassium (63.72 ± 1.67 mg/100 g), calcium (115.60 ± 2.93 mg/100 g), magnesium (28.84 ± 1.08 mg/100 g), and iron (12.12 ± 0.69 mg/100 g). The plant also contains copper, zinc, and manganese. Furthermore, it has low levels of lead (0.0263 ± 0.0010 µg/g), arsenic (0.95 ± 0.0007 µg/g), and mercury (0.14 ± 0.002 µg/g), which are within safe limits for human consumption (Table 1).

The potassium content in *M. bengalensis* is higher than that of vegetables like lettuce (33 mg/100 g), beetroot (43 mg/100 g), and cucumber (50 mg/100 g), but lower than carrots (108 mg/100 g) and bottle gourd (87 mg/100 g). The favorable potassium-to-sodium (K/Na) ratio suggests its potential in managing hypertension and promoting healthy blood pressure levels¹⁴.

In terms of calcium, *M. bengalensis* (115.60 mg/100 g) provides more than many common vegetables like lettuce (36 mg/100 g), broccoli

(47 mg/100 g), and potato leaves (120 mg/100 g), though it is slightly lower than spinach (136 mg/100 g) and soya leaves (180 mg/100 g). This makes it a valuable source of calcium for bone health and cardiac function^{14,16-18}.

The iron content in *M. bengalensis* (12.12 mg/100 g) is higher than that of soya leaves (8 mg/100 g) and sweet potatoes (10 mg/100 g), but lower than neem leaves (17.1 mg/100 g), soya beans (15.7 mg/100 g), and amaranth seeds (11 mg/100 g). This significant iron level makes it beneficial in preventing anaemia and supporting oxygen transport in the body^{14,16-18}.

Regarding zinc, *M. bengalensis* contains modest amounts compared to high-zinc sources like pumpkin seeds (7.8 mg/100 g), sesame seeds (7.8 mg/100 g), and chickpeas (4.6 mg/100 g), but more than vegetables like asparagus (0.54 mg/100 g) and betel leaves (3.44 mg/100 g). Zinc is crucial for supporting metabolic processes and immune function^{14,16-18}.

Overall, the mineral content of *M. bengalensis* highlights its nutritional value as a good source of essential minerals, while its low levels of harmful metals ensure its safety for regular consumption, promoting both health benefits and minimal risk of toxicity¹⁶⁻¹⁸.

Water soluble vitamins in *M. bengalensis* by HPLC

The HPLC method used in this study accurately determined the content of water-soluble vitamins, including vitamin C and various B vitamins, in *M. bengalensis*. The chromatograms in (Fig. 1) and (Fig. 2) confirmed the presence of these vitamins in the plant. The quantitative analysis, expressed as mg/100 g of dry plant material in Table 1, revealed significant amounts of these vitamins in *M. bengalensis*.

M. bengalensis exhibited a high concentration of vitamin C, measuring 93.67 ± 4.12 mg/100 g, which is significantly higher than that found in many common vegetables. For instance, spinach contains approximately 28 mg/100 g, and cauliflower provides about 56 mg/100 g¹⁹. It also surpasses carrot leaves (79 mg/100 g) and beet greens (70 mg/100 g), and is close to vegetables like bitter melon (96 mg/100 g) and amaranth stems (99 mg/100 g)¹⁴. However, *M. bengalensis* is surpassed by certain wild vegetables, such as kakrol (*Coccinia grandis*), which can contain up to 150 mg/100 g, and *Moringa* leaves, which may provide as much as 200 mg/100 g¹⁹.

The high vitamin C content in *M. bengalensis* suggests its potential to prevent scurvy, promote

healthy skin, and support collagen synthesis. Including this wild plant in the diet can provide a significant portion of the daily vitamin C intake, boosting immunity and reducing the risk of chronic conditions like atherosclerosis and certain cancers⁹. This positions *M. bengalensis* as a rich source of vitamin C, comparable to the highest vitamin C-containing plants, contributing to overall well-being with strong antioxidant support.

The HPLC analysis also detected several B vitamins in *M. bengalensis*, including vitamin B1 (3.74 ± 0.16 mg/100 g), B2 (0.24 ± 0.02 mg/100 g), B3 (0.27 ± 0.02 mg/100 g), B5 (3.61 ± 0.002 mg/100 g), B6 (6.75 ± 0.44 mg/100 g), and vitamin B9 (18.49 ± 1.52 µg/100 g).

The vitamin B1 content in common vegetables is relatively low, as compared to spinach containing approximately 0.08 mg/100 g and potatoes around 0.1 mg/100 g¹⁹. In contrast, certain wild vegetables, such as amaranth leaves, can have significantly higher

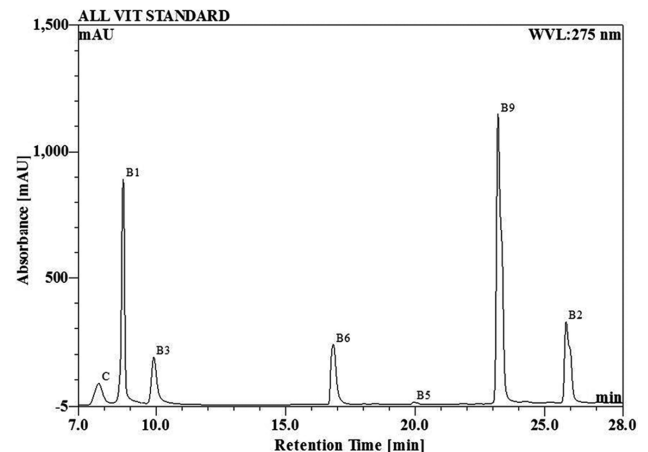


Fig. 1 — HPLC Chromatogram of mixture of Standard vitamin

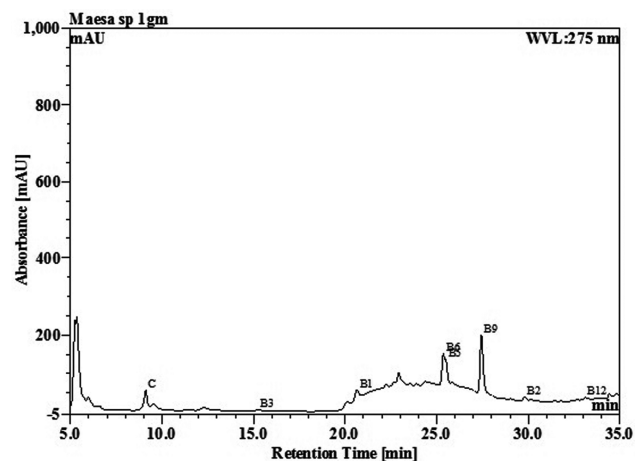


Fig. 2 — HPLC Chromatogram of *Maesa bengalensis* for vitamin

vitamin B1 levels, reaching up to 5.0 mg/100 g²⁰. This stark difference highlights the potential of wild plants as rich sources of essential vitamins compared to commonly consumed vegetables.

M. bengalensis contains 0.24±0.02 mg/100 g of vitamin B2, similar to spinach (0.25 mg/100 g) and comparable to asparagus (0.28 mg/100 g) and broccoli (0.4 mg/100 g). It contains more vitamin B2 than vegetables like lettuce (0.04 mg/100 g), cucumber (0.03 mg/100 g), and potato (0.02 mg/100 g). While lower than some vegetables, its B2 content supports energy metabolism and maintains healthy skin and eyes^{14,19}.

M. bengalensis contains 0.27±0.02 mg/100 g of vitamin B3, comparable to vegetables like celery (0.3 mg/100 g) and bitter melon (0.4 mg/100 g), but higher than cucumber (0.2 mg/100 g) and green papaya (0.1 mg/100 g). While lower than lettuce and spinach (0.5 mg/100 g), it still provides a valuable source of niacin, essential for metabolism, skin, nerve, and digestive health. Incorporating it into the diet helps meet daily niacin needs, especially when combined with other nutrient-rich vegetables^{14,19}.

The vitamin B9 content in *M. bengalensis* is 18.49±1.52 µg/100 g, which is notable when compared to other vegetables. While it falls short of spinach, a concentrated source of folate at 194 µg/100 g, *M. bengalensis* still provides a comparable amount to several common vegetables. For instance, cabbage contains 23 µg/100 g, brinjal provides 34 µg/100 g,

and plantain green offers 16.4 µg/100 g. Other vegetables, such as carrot (15 µg/100 g), yam (17.5 µg/100 g), snake gourd (15.5 µg/100 g), and pumpkin (13 µg/100 g), have folate levels slightly lower or similar to *M. bengalensis*^{14,19}.

Though not as rich in folate as spinach, *M. bengalensis* still contributes significantly to dietary folate intake, which is essential for DNA synthesis, cell division, and overall health, making it a beneficial addition to the diet

Quantification of amino acids in *M. bengalensis* by HPLC

The amino acid profiles of three *M. bengalensis* leaf extracts were determined by HPLC (Table 2). Seventeen amino acids were identified, including eight essential, one semi-essential, and eight non-essential amino acids. The HPLC method was validated, ensuring reliable comparison of amino acid content across extracts.

The water extract of *M. bengalensis* contained notable amounts of L-glutamic acid (1.34 µg/mg), L-arginine (1.28 µg/mg), and L-aspartic acid (0.74 µg/mg). L-glutamic acid aids metabolism and boosts antitumor drug efficacy. L-arginine supports cardiovascular health, tissue repair, and immunity, while L-aspartic acid has food and pharmaceutical uses²¹⁻²⁴.

The water extract had the highest L-histidine content (2.68 µg/mg), exceeding levels in common vegetables. L-histidine is crucial for infant nutrition

Table 2 — Quantification of amino acids in *Maesa bengalensis* by HPLC

| | Amino acid | Water extract (µg/mg) | Methanol extract (µg/mg) | Benzene extract (µg/mg) |
|-----|-------------------------|-----------------------|--------------------------|-------------------------|
| 1. | L-Aspartic acid | 0.74±0.07 | 0.34±0.09 | 0.11±0.009 |
| 2. | L-Glutamic acid | 1.34±0.14 | 0.28±0.06 | 0.09±0.002 |
| 3. | L-Serine | 0.59±0.06 | 0.16±0.008 | ND |
| 4. | L-Histidine * | 2.68±0.76 | 1.11±0.29 | 0.19±0.004 |
| 5. | Glycine | 0.23±0.06 | 0.08±0.001 | ND |
| 6. | L-Threonine* | 0.67±0.09 | 0.32±0.03 | 0.11±0.004 |
| 7. | L-Arginine | 1.28± 0.34 | 0.76±0.09 | 0.18±0.005 |
| 8. | L-Alanine | 0.47±0.06 | 0.09±0.006 | ND |
| 9. | L-Tyrosine | 0.56± 0.05 | 0.18±0.09 | ND |
| 10. | L-Cysteine | 0.34±0.06 | 0.07±0.003 | ND |
| 11. | L-Valine* | 0.88±0.03 | 0.41±0.09 | 0.08±0.001 |
| 12. | L-Methionine* | 0.352±0.09 | 0.11±0.06 | 0.04±0.001 |
| 13. | L-Phenylalanine* | 0.81±0.07 | 0.32±0.005 | 0.11±0.07 |
| 14. | L-Isoleucine* | 0.64±0.08 | 0.26±0.03 | ND |
| 15. | L-Leucine* | 1.06±0.27 | 0.68±0.11 | 0.22±0.08 |
| 16. | L-Lysine hydrochloride* | 0.87±0.07 | 0.34±0.05 | 0.09±0.005 |
| 17. | L-Proline | 0.46±0.08 | 0.12±0.05 | ND |

ND: Not detected; * essential amino acid

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

and aids in managing plasma lipids, insulin resistance, and inflammation²¹. L-leucine, an essential amino acid detected in all extracts, supports energy metabolism and cellular signalling, with potential benefits for obesity and diabetes²². L-phenylalanine, highest in the water extract (0.81 µg/mg), exhibits antidepressant effects and diverse industrial applications²³. L-isoleucine, found in water and methanol extracts, holds nutritional and therapeutic importance, while L-lysine (0.16-0.59 µg/mg) contributes to energy metabolism and cholesterol reduction²⁴. L-serine, vital for cellular proliferation and brain function, was present in similar amounts (0.16-0.59 µg/mg) in both extracts²⁵.

The amino acid profile of *M. bengalensis* highlights its nutritional value, offering essential, nonessential, and semi-essential amino acids that support protein synthesis and overall health.

Antioxidant activities of *M. bengalensis* in three different solvent extracts

In this study, the antioxidant activities of benzene, methanol, and water extracts of *M. bengalensis* were assessed (Table 3). The total phenolic content ranged from 8.50±0.68 to 36.08±3.09 mg GAE/100 g of dry plant material and the water extract exhibited the highest flavonoid content (19.94±2.35 mg RE/100 g). These findings suggest that *M. bengalensis* leaves have substantial antioxidant potential and can serve as natural sources of antioxidants.

The metal chelating activity of *M. bengalensis* extracts was assessed, with the water extract showing the highest activity (41.65±2.77%), followed by methanol (31.28±4.67%), and benzene showing the least. Metal ions can trigger lipid peroxidation, contributing to conditions like cancer and joint pain¹¹. The observed chelating activity suggests the plant's potential in preventing oxidative damage and treating metal ion-induced ailments¹¹.

Anti-lipid peroxidation analysis showed the water extract of *M. bengalensis* had the highest inhibition

(23.56±1.94%), indicating strong antioxidant activity. The benzene fraction exhibited the least inhibition, suggesting weaker antioxidant effects. These differences highlight the importance of solvent selection in extraction, as it impacts the efficiency and composition of bioactive compounds, including antioxidants¹⁰.

The study highlights the water extract of *M. bengalensis* as a potent lipid peroxidation inhibitor. Further research is needed to identify the bioactive compounds responsible for this effect. Understanding the mechanisms involved can help develop natural antioxidants for oxidative stress-related diseases. Notably, the water extract showed the highest lipid peroxidation inhibition, emphasizing its potential for addressing oxidative stress disorders¹¹.

Identification and quantification of phenolic acids and flavonoids in *M. bengalensis* by HPLC

This study evaluated the antioxidant properties of benzene, methanol, and water extracts of *M. bengalensis*. RP-HPLC analysis identified 13 phenolic acids and 8 flavonoids. The concentrations of these compounds varied across extracts, as shown in Table 4 and Figure 3 a,b,c.

The water extract of *M. bengalensis* showed high gallic acid (0.15±0.07 µg/mg) and protocatechuic acid (0.41±0.03 µg/mg) levels, contributing to its antioxidant properties and potential against oxidative stress-related diseases²⁶⁻²⁷.

Chlorogenic acid in the methanol extract (1.11±0.68 µg/mg) may help lower blood sugar and exhibit anti-diabetic properties²⁸. Its presence suggests a link between *M. bengalensis* consumption and reduced risk of liver cirrhosis and cancer²⁹.

Syringic acid, found in the highest concentration in the water extract (18.01±1.11 µg/mg dry extract), is known for its anti-cancer, anti-proliferative, and hepatoprotective properties³⁰. Ferulic acid, detected in the range of 0.067±0.002-4.23±0.73 µg/mg dry extract, has various physiological functions, including

Table 3 — Antioxidant properties of *Maesa bengalensis*

| Antioxidant parameter | Benzene extract | Methanol extract | Water extract |
|---|-----------------|------------------|---------------|
| Total phenolic content (GAE mg/100 g dry plant material) | 8.50±0.68 | 29.77±1.27 | 36.08±3.09 |
| Total flavonoid content (Rutin equivalent mg/100 g dry plant material) | 1.41±0.89 | 7.90±0.72 | 19.94±2.35 |
| Total reducing power (Ascorbic acid equivalent mg/100 g dry plant material) | 3.06±0.66 | 6.46±0.96 | 8.84±0.87 |
| DPPH radical scavenging activity (IC ₅₀ mg dry plant material) | 9.64±0.59 | 2.84±0.61 | 2.74±0.77 |
| ABTS radical scavenging activity (IC ₅₀ mg dry plant material) | 1.60±0.38 | 0.49±0.09 | 0.33±0.08 |
| Metal chelating activity % of inhibition | 12.24±1.11 | 31.28±4.67 | 41.65±2.77 |
| Lipid peroxidation assay (% of inhibition) | 6.53±0.59 | 15.89±2.96 | 23.56±1.94 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

Table 4 — Quantification of phenolic acids and flavonoids in *Maesa bengalensis* by HPLC in three different solvent extract

| Phenolic acids/Flavonoids | Benzene extract ($\mu\text{g}/\text{mg}$ dry extract) | Methanol extract ($\mu\text{g}/\text{mg}$ dry extract) | Water extract ($\mu\text{g}/\text{mg}$ dry extract) |
|--------------------------------|--|---|--|
| Gallic acid | 0.008 \pm 0.0003 | 0.032 \pm 0.006 | 0.15 \pm 0.07 |
| Protocatechuic acid | ND | 0.24 \pm 0.08 | 0.41 \pm 0.03 |
| Gentisic acid | ND | ND | ND |
| <i>p</i> -Hydroxy benzoic acid | ND | 0.062 \pm 0.005 | 0.45 \pm 0.09 |
| Chlorogenic acid | 0.043 \pm 0.002 | 1.11 \pm 0.68 | 0.04 \pm 0.001 |
| Vanillic acid | ND | ND | ND |
| Caffeic acid | 0.088 \pm 0.007 | ND | ND |
| Syringic acid | 0.059 \pm 0.005 | 2.73 \pm 0.52 | 18.01 \pm 1.11 |
| <i>p</i> -Coumaric acid | 0.051 \pm 0.006 | 0.19 \pm 0.08 | 0.013 \pm 0.008 |
| Ferulic acid | 0.067 \pm 0.002 | 0.37 \pm 0.07 | 4.23 \pm 0.73 |
| Sinapic acid | 0.047 \pm 0.005 | 0.088 \pm 0.009 | 0.29 \pm 0.04 |
| Salicylic acid | ND | ND | ND |
| Ellagic acid | 0.349 \pm 0.07 | 1.19 \pm 0.38 | 4.24 \pm 0.52 |
| Naringin | 0.087 \pm 0.006 | ND | ND |
| Rutin | 0.345 \pm 0.09 | 0.51 \pm 0.02 | 0.55 \pm 0.009 |
| Myricetin | 0.235 \pm 0.04 | 0.29 \pm 0.06 | 0.37 \pm 0.03 |
| Quercetin | 0.653 \pm 0.08 | 4.42 \pm 0.66 | 37.56 \pm 1.06 |
| Naringenin | 0.038 \pm 0.008 | 0.70 \pm 0.06 | 3.69 \pm 0.68 |
| Apigenin | 0.086 \pm 0.007 | 1.56 \pm 0.72 | 3.51 \pm 0.23 |
| Kaempferol | 3.70 \pm 0.49 | 5.14 \pm 0.71 | 7.05 \pm 0.44 |
| Catechin | ND | 1.30 \pm 0.29 | 2.72 \pm 0.33 |

ND: Not detected

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean \pm SEM

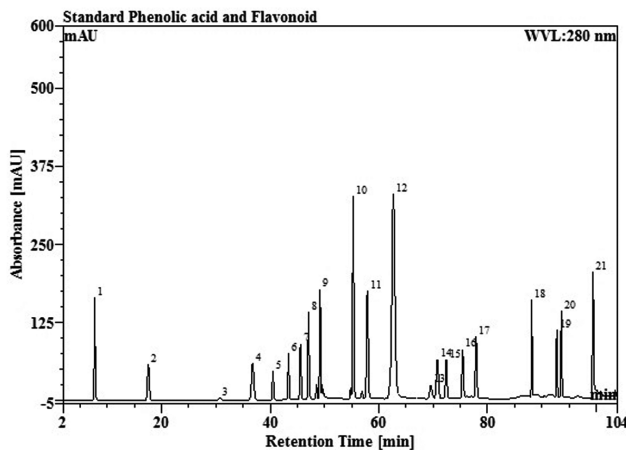


Fig. 3 — HPLC Chromatogram of mixture of Standard phenolic acids and flavonoids 1. Gallic acid, 2. Protocatechuic acid, 3. Gentisic acid, 4. *p*-Hydroxy benzoic acid, 5. Catechin, 6. Chlorogenic acid, 7. Vanillic acid, 8. Caffeic acid, 9. Syringic acid, 10. *p*-Coumaric acid, 11. Ferullic acid, 12. Sinapic acid, 13. Salicylic acid, 14. Naringin, 15. Rutin, 16. Ellagic acid, 17. Myricetin, 18. Quercetin, 19. Naringenin, 20. Apigenin, 21. Kaempferol

antimicrobial, anti-inflammatory, anti-diabetic, and anti-cancer activities³¹.

Quercetin, abundant in various fruits and vegetables, was present in significant amounts in the water extract (37.56 \pm 1.06 $\mu\text{g}/\text{mg}$ dry extract),

followed by the methanol extract (4.42 $\mu\text{g}/\text{mg}$ dry extract) and it is known for its anti-cancer, anti-histamine, and anti-inflammatory properties^{32,33}. Kaempferol, found in the water extract (7.05 \pm 0.44 $\mu\text{g}/\text{mg}$ dry extract), has been associated with the prevention of low-density lipoprotein oxidation and a lower risk of stomach cancer³⁴.

Ellagic acid, myricetin, and apigenin were abundant in methanol and water extracts, with therapeutic potential for depression, cancer, infections, liver, and cardiovascular diseases^{35,36}.

The phenolic acids and flavonoids in *M. bengalensis* extracts exhibit antioxidant, anti-inflammatory, and anti-cancer properties, highlighting its potential for therapeutic uses and functional foods.

Anti-nutritional composition

The anti-nutrient analysis (Table 1) showed *M. bengalensis* contains 0.24 \pm 0.08% oxalate, similar to common foods like spinach (0.66%), almonds (0.41%), amla (0.29%), and amaranth (0.77%)¹⁴. Oxalate can bind to minerals and may lead to mineral deficiencies^{37,38}.

Phytic acid, another anti-nutrient, forms insoluble complexes with minerals, reducing their bioavailability³⁹. The plant under study had a phytic

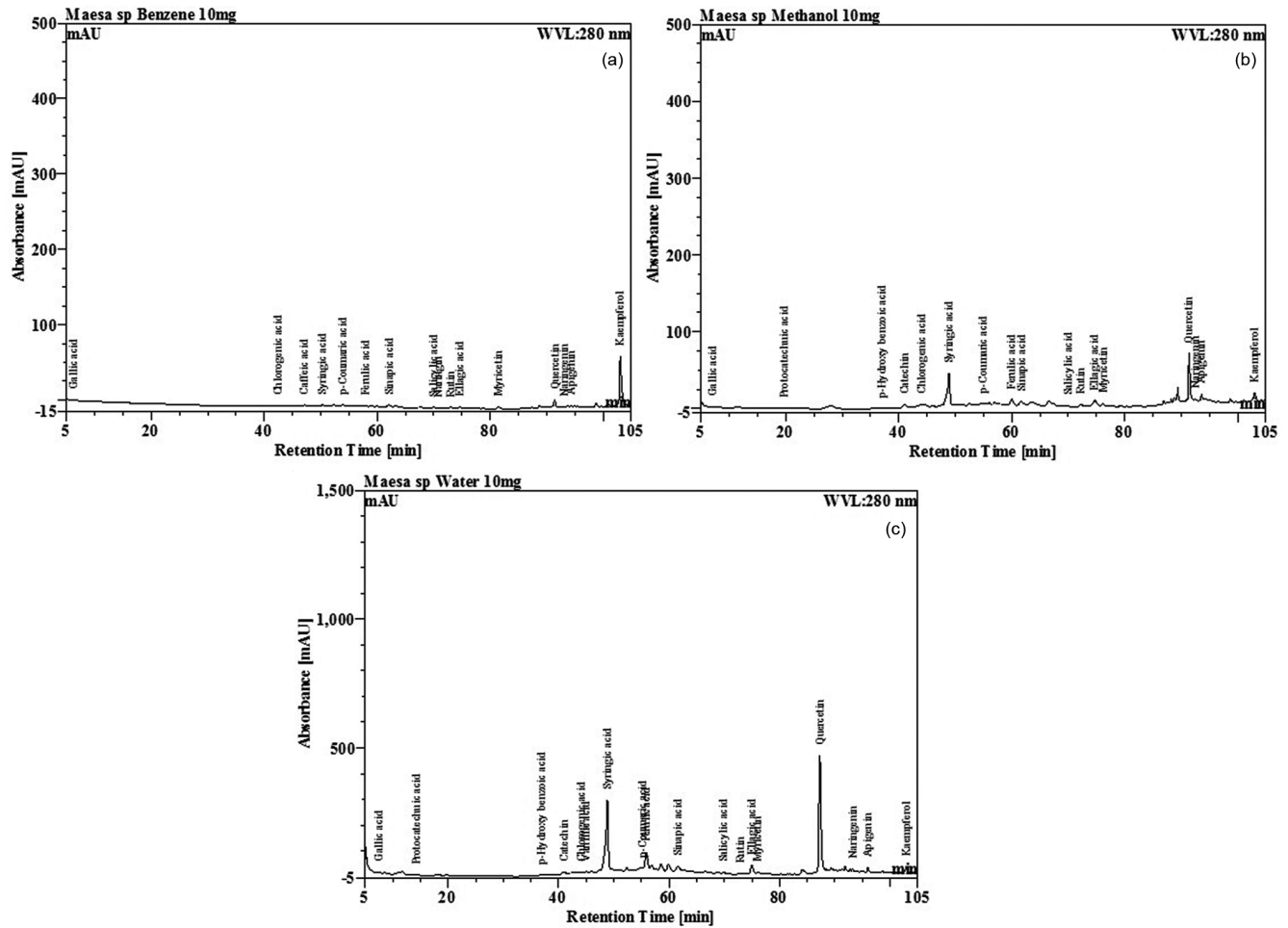


Fig. 3 (a-c) — (a) HPLC Chromatogram of benzene extract of *Maesa bengalensis*, (b) HPLC Chromatogram of methanol extract of *Maesa bengalensis* & (c) HPLC Chromatogram of water extract of *Maesa bengalensis*

acid level of $0.21 \pm 0.03\%$, which is lower than the range of 10–60 mg/g, that requires attention for mineral bioavailability challenges^{40,41}. Saponins, which can cause red blood cell damage and interfere with thyroid function, were present in the plant at a low level of $0.16 \pm 0.09\%$, indicating their safe consumption⁴². Tannins, significant anti-nutritional components, can hinder enzyme functions and impair iron absorption³⁹. The low concentration of tannins suggests no harmful effects on humans. Cyanogenic glycosides are present in various plants, and their hydrolysis releases hydrogen cyanide, which can cause cyanide poisoning⁴³. The investigation found low levels of cyanide content in the plant, confirm its safety for consumption, making it a nutritious and antioxidant-rich wild edible.

Toxicity studies

Haemolytic tests assessed the safety of *M. bengalensis* aqueous extracts using rat erythrocytes at

various concentrations (100–1000 $\mu\text{g/mL}$) with H_2O_2 as a positive control (Table 5). The extract showed high cell viability ($90.55 \pm 1.08\%$) compared to 51.75% for H_2O_2 -treated cells and 100.88% for the buffer control, confirming minimal effects on red blood cells and supporting its safety⁴⁴.

Cytotoxicity studies were conducted using isolated hepatocytes from fresh goat liver. Hepatocyte viability was assessed with plant extract concentrations (100–1000 $\mu\text{g/mL}$). At 1000 $\mu\text{g/mL}$, viability was $88.25 \pm 1.05\%$, comparable to the control, while H_2O_2 -treated cells (200 μM) showed $41.25 \pm 1.05\%$ (Table 5). The high viability confirms the extract's non-toxic nature and safety for consumption⁴⁵.

The genotoxicity of the aqueous extract was evaluated using the Comet assay (Table 5) and (Fig. 4). Even at a high concentration of 1000 $\mu\text{g/mL}$, the extract exhibited low olive tail moment (OTM)

| | Viability of rat RBC cell with plant extract (1000 µg/mL) | Viability of rat RBC cell with Phosphate buffer (Negative control) | Viability of rat RBC cell with H ₂ O ₂ (200 µM) (Positive control) |
|----------------------------|---|--|--|
| Haemolytic toxicity | 90.55±1.08% | 100.88 ± 1.03% | 51.75 ± 1.28% |
| | Viability of goat liver hepatocytes with plant extract (1000 µg/mL) | Viability of goat liver hepatocytes with Phosphate buffer (Negative control) | Viability of goat liver hepatocytes with H ₂ O ₂ (200 µM) (Positive control) |
| Cytotoxicity (MTT assay) | 88.25±1.05% | 99.56±0.56% | 41.25±1.05% |
| | DNA damage index (OTM) with plant extract (1000 µg/mL) | DNA damage index (OTM) with Phosphate buffer (Negative control) | DNA damage index (OTM) with H ₂ O ₂ (200 µM) (Positive control) |
| Genotoxicity (Comet assay) | 2.11±0.35 | 1.79±0.36 | 21.38±1.98 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

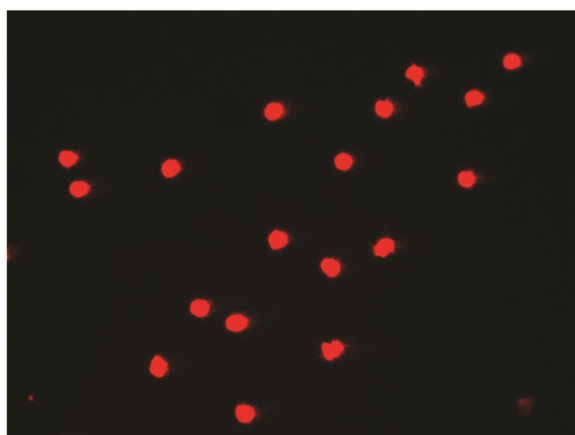


Fig. 4 — Comet image of *Maesa bengalensis*

values of 2.11±0.35, indicating minimal DNA damage. The negative control group had an OTM value of 1.79±0.36, while the positive control (mixture of whole blood, RPMI 1640, and 200 µM H₂O₂) showed a significantly higher OTM of 21.38±1.98. These results suggest that the plant extract induces minimal genotoxic effects and is comparable to the baseline DNA damage in living organisms. The findings support the safety of consuming *M. bengalensis*⁴⁶.

Haemolytic, cytotoxicity, and genotoxicity assessments confirm the non-toxic nature of *M. bengalensis* aqueous extracts at cellular and genomic levels, supporting its safe consumption. Further *in vivo* studies are needed to confirm its safety and health benefits.

Conclusion

This study highlights the nutritional and health benefits of *M. bengalensis* leaves, making them a valuable addition to human diets. The plant is rich in essential minerals, free amino acids like L-histidine, and phenolic compounds such as quercetin, which contribute to its potent antioxidant activity. It is low

in antinutrients and offers protein, fiber, calcium, and carbohydrates, supporting its potential as a nutrient-dense food. Toxicity studies confirm its safety, aligning with the traditional practices of the Mao Naga tribe.

The evergreen nature and ease of propagation through vegetative cuttings make *M. bengalensis* a reliable green vegetable, especially during the dry season, and economically beneficial for subsistence farmers. Promoting its use in Indian diets and as a superfood supplement could help combat malnutrition-related diseases.

Addressing its genetic diversity is crucial for conservation and sustainable use, as it can provide insights into the plant's adaptability, nutritional variation, and domestication potential. Further research is necessary to explore its therapeutic properties and optimize its cultivation for broader health and economic benefits.

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Conflict of Interest

We declare that we have no conflict of interest.

Author Contributions

AAM, carried out the ethnobotanical studies of the Mao Naga tribe and edited the manuscript. TS, carried out the phytochemical analyses of the plant and drafted the mss. GK, carried out the taxonomical studies of the plant.

Ethical Statements

This research article is exempt because no human participant was recruited.

Data Availability

The authors approved that the data supporting the result and finding of this experiment are available from the corresponding author upon request.

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