



Biochemical profiling of steamed leaf extracts used in traditional food wrapping

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Around the world, various materials have been used for food packaging and wrapping, but natural food wrappers are now gaining popularity due to their perceived safety and the presence of beneficial phytochemicals having health benefits. In this study, comparative biochemical profiling of extracts from steamed leaves was performed, which are traditionally used by tribal community of Arunachal Pradesh for food wrapping during various food preparations through steaming. The leaves studied included *Mallotus tetracoccus*, *Phrynium capitatum*, *Musa acuminata*, *Curcuma longa* and *Amomum subulatum*. A total of eighteen phenolic acids, thirteen flavanoids, eleven organic acids and seven water soluble vitamins were identified in the leaf extract. It was found that, the extract of *Mallotus tetracoccus* followed by *Musa acuminata* had highest content of phenolic acids, flavanoids, organic acids and vitamins. Gallic acid (10.79 µg/mL) was recorded highest in *Mallotus tetracoccus* and caffeic acid (6.29 µg/mL) in *Musa acuminata* among the phenolic acids. *Mallotus tetracoccus* was found to contain highest amount of catechin (16.56 µg/mL) and shikimic acid (1217.83 µg/mL) among flavonoids and organic acids, respectively. Vitamin B₃ (niacin), a water-soluble vitamin was also found highest in *Mallotus tetracoccus* (31.83 ng/mL) followed by *Musa acuminata* (19.23 ng/mL). Thus, it has been concluded that, steaming or wrapping the warm food with the leaves of *Mallotus tetracoccus* and *Musa acuminata* not only provides an alternative for health detrimental synthetic food wrapper but also supplements the food with several health beneficial bio-active compounds.

Keywords: Bio-active compounds, Biodegradable, Carcinogenic, Plastic

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Natural food wrappers were used in India and around the world before the discovery of plastic wrapper. Traditionally, leaves were used as food wrappers in many countries^{1,2}. The shift towards plastic food wrapper from natural food wrapper in today's daily life has significantly affected the environment and human health. Plastic materials are not easily degradable, thereby contributing to environment pollution. Plastic and plastic derivatives liberates various harmful chemicals such as, bisphenol A, phthalates, melamine etc. into the food during usage which are potentially carcinogenic (prostate and breast cancer), insulin resistance and cause heart diseases³. The compound melamine is toxic and cause kidney failure and stone formation in kidneys⁴. Polystyrene, one of the widely used plastics is carcinogenic and also affects the thyroid hormone level⁵. Additionally, use of plastic results in microplastics pollution which is due to long-lasting, importunate, slow-degrading plastic fragments

that gets integrated in the food chain system. As per the DNA (daily news and analysis) India report, people in modern day swallow two polythene bags per year in the form of microplastics⁶. The reports regarding accumulation of microplastics in animal and human organs and even in the mother's milk has made it essential to search for an effective biodegradable alternative⁷. The replacement of the plastic disposables & food wrapping materials by the traditionally and eco-friendly used organic food wrappers (leaves) can be one of the best alternative and an important step in combating environmental and health hazard.

Since ages, the Southeast Asian countries including several tribes in India use various leaves such as, *Mallotus tetracoccus* (Roxb.) Kurz., *Phrynium capitatum* Willd., *Musa acuminata* L., *Curcuma longa* L. and *Amomum subulatum* Roxb. for food wrapping. These leaves were reported to comprise phytochemicals of health benefiting properties. *Mallotus tetracoccus* leaf extracts was reported to have antioxidant properties. Several reports have been published on various

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therapeutic properties of *Mallotus* genus *viz.*, antipyretic, hepatoprotective and anti-inflammatory⁸. Gautam *et al.*⁹ reported that aqueous and alcoholic extract of *A. subulatum* have analgesic, antimicrobial, antioxidative, cardio-protective, hypolipidemic and anti-inflammatory properties. *P. capitatum* leaves have reported to contain phytochemicals belongs to alkaloid, flavonoid, steroid, phenolics etc.¹⁰ The plant extracts of *M. acuminata* and *C. longa* have antimicrobial properties^{11,12}. Furthermore, *C. longa* is well known crop with many bio-activities of health importance such as, free radical scavenging, anti-inflammatory, hypoglycemic etc.¹² Leaves when used for steaming and food wrapping is known to release several bioactive compounds directly into the food which have health benefiting activities. Leaves like turmeric and banana also imparts characteristic flavors to the food cooked in them² (Fig. 1). However, till date no research has been carried out to find out the compounds released from these leaves while steaming or serving warm food on them. Hence, the present research was carried out to profile the phytochemicals released from leaves upon steaming which will helps to decipher the efficacy of the traditionally used leaves as an alternative to plastic or other synthetic wrapper. As per our knowledge, this is the first report on functional metabolites present in the steamed leaf extracts.

Materials and Methods

Sample preparation

Leaf sample of 10 cm² of each plant species *viz.*, *Phrynium capitatum* (2.34 g), *Musa acuminata* (2.28 g), *Amomum subulatum* (2.18 g), *Curcuma longa* (1.41 g), *Mallotus tetracoccus* (2.29 g) were collected from the College of Horticulture and Forestry, Pasighat, Arunachal Pradesh, India (Fig. 2). Collected leaves were steamed in an autoclave without tightening lid at 100°C for 15 min. by keeping in a glass beaker containing 10 mL of water in petridish. The cooled steamed extracts were collected and mixed with aqueous methanol to make up 25 mL which was further used for estimation.

Estimation of total phenol and flavonoid

Total phenolic and flavanoid contents in steamed leaf extracts were determined to follow the methods performed by Singleton *et al.*¹³ and Chun *et al.*¹⁴, respectively.

Chemicals and reagents

All the standards used in this study were acquired from Sigma-Aldrich, St. Louis, Missouri, USA. The reagents used for chromatographic analysis were of MS-grade while all the other solvents were of



Fig. 1 — (a) Leaves of *Phrynium capitatum* Willd. (b) *Curcuma longa* L. and (c) *Musa acuminata* L. used for food wrapping and steaming

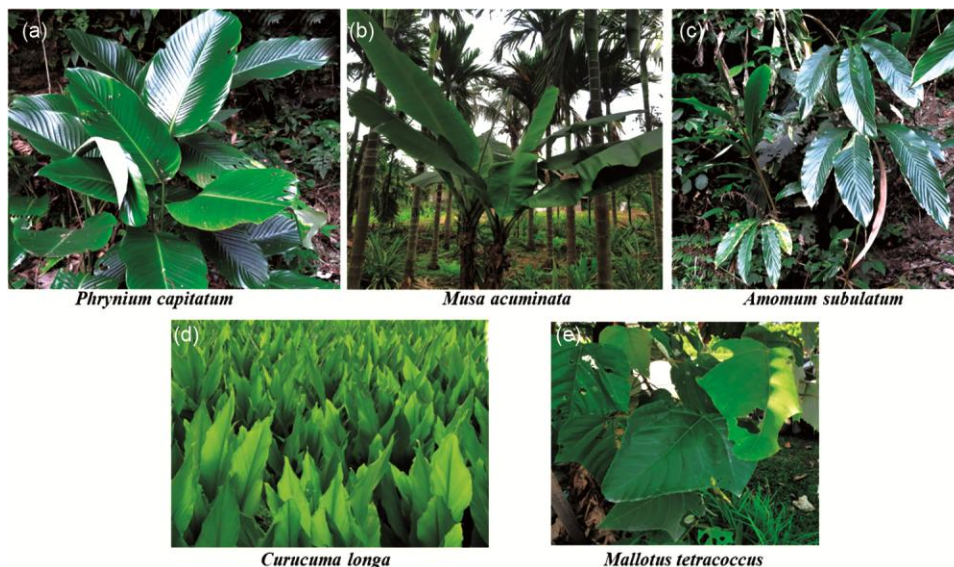


Fig. 2 — Leaves used for food steaming and wrapping (a) *Phrynium capitatum* Willd. (b) *Musa acuminata* L. (c) *Amomum subulatum* Roxb. (d) *Curcuma longa* L. (e) *Mallotus tetracoccus* (Roxb.) Kurz

analytical grade. Ultra-purified water after passing it through 0.2 μm membrane filter was used for the preparation of HPLC mobile phase.

Profiling of phenolic acids, flavonoids, organic acids and vitamins (water-soluble) using UPLC-MS/MS

An UPLC-MS/MS system Acquity UPLC-H Class equipped with TQD-MS/MS (waters Inc., USA) was used to carry out the profiling of phenolic acids, flavonoids, organic acids and water-soluble vitamins. Analysis was carried out through MasslynxTM software connected with the system.

Waters UPLC BEH-C18 column (2.1 \times 50 mm dimension and 1.7 μm particle size) was employed to profile phenolic acids, flavanoids and water soluble vitamins while 2.1 \times 100 mm UPLC BEH-Amide column (particle size of 1.7 μm , Waters, USA) was used for organic acid profiling. The UPLC temperature control compartment was uphold at 25 \pm 1 $^{\circ}\text{C}$.

Calibration curve

The calibration curves were prepared through the multiple reactions monitoring (MRM) method of UPLC-MS/MS for each of the compounds to be analysed. This was done using the parent mass (m/z) and most abundant fragmented daughters.

Profiling of phenols and flavonoids

Sample isolation for LC-MS/MS based profiling of phenolics and flavanoids for the analysis were carried out as mentioned by Chen *et al.*¹⁵. After extraction, the supernatant (ethyl acetate) was collected and dried

completely using rotary evaporator. Methanol (1 mL) was poured into the dried sample filtered through 0.2 μm nylon filter followed by injection into the LC-MS/MS system (Waters, USA). The phenolic and flavonoid profiling was done precisely using methodology followed by Arivalagan *et al.*¹⁶ and Bhargav *et al.*¹⁷. The MRM details for phenolics and flavanoids are presented in the Supplementary Table S1 and S2, respectively.

Profiling of organic acids

Organic acids' extraction was performed following the methods described by Oliveira *et al.*¹⁸ and Ribeiro *et al.*¹⁹, with minor modifications. The eluted volume was made up to 1 mL with the mobile phase, consisting of solvent A (ammonium acetate of 10 mM and acetonitrile in the ratio of 50:50 having pH 8.5) and Solvent B (acetonitrile containing 0.05% formic acid). 2 μL volume phase was injected into the LC-MS/MS system. Elution conditions for organic acids profiling was similar, as described by Arivalagan *et al.*¹⁶, and Yattung *et al.*²⁰ The MRM details for each compound are included in Supplementary Table S3.

Profiling of water soluble vitamins

Water soluble vitamins were profiled using the method described by Arivalagan *et al.*¹⁶. The MRM details are given in Supplementary Table S4.

Statistical analysis

All the ultimate data are presented as mean \pm standard deviation on fresh weight basis, where n= 3.

Table 1 — Estimation of total Phenol and flavonoid ($\mu\text{g/mL}$)

Parameters	<i>Phrynium capitatum</i>	<i>Musa acuminata</i>	<i>Amomum subulatum</i>	<i>Curcuma longa</i>	<i>Mallotus tetracoccus</i>
Total Phenols	7.56 \pm 0.06	32.32 \pm 0.84	4.81 \pm 0.06	8.62 \pm 0.26	50.01 \pm 1.10
Total Flavonoids	3.38 \pm 0.42	18.89 \pm 0.73	1.69 \pm 0.10	2.21 \pm 0.21	25.14 \pm 1.25

Table 2 — Phenolic acids and flavonoid profiling of steamed leaf extracts ($\mu\text{g/mL}$)

	<i>Phrynium capitatum</i>	<i>Musa acuminata</i>	<i>Amomum subulatum</i>	<i>Curcuma longa</i>	<i>Mallotus tetracoccus</i>
<i>Phenolic acids</i>					
Benzoic acid	0.02 \pm 0.01	0.05 \pm 0.01	0.01 \pm 0.00	0.03 \pm 0.00	0.04 \pm 0.00
Salicylic acid	0.84 \pm 0.05	3.69 \pm 0.08	0.32 \pm 0.01	0.78 \pm 0.02	7.94 \pm 0.04
3-Hydroxy benzoic acid	0.00 \pm 0.00	0.01 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00
t-Cinnamic acid	0.42 \pm 0.01	4.02 \pm 0.04	0.62 \pm 0.05	1.33 \pm 0.08	8.74 \pm 0.17
2,4-Dihydroxy benzoic acid	0.19 \pm 0.01	0.17 \pm 0.01	0.10 \pm 0.01	0.19 \pm 0.01	0.92 \pm 0.01
Gentisic acid	0.57 \pm 0.05	0.93 \pm 0.02	0.12 \pm 0.02	1.02 \pm 0.07	1.33 \pm 0.02
Protocatechuic acid	0.47 \pm 0.03	0.82 \pm 0.05	0.22 \pm 0.03	0.74 \pm 0.06	2.87 \pm 0.10
3,4-Dihydroxy benzoic acid	0.17 \pm 0.01	0.20 \pm 0.00	0.11 \pm 0.02	0.27 \pm 0.01	0.26 \pm 0.02
o-Coumaric acid	0.09 \pm 0.01	0.23 \pm 0.02	0.09 \pm 0.01	0.37 \pm 0.04	1.52 \pm 0.01
p-Coumaric acid	0.30 \pm 0.04	0.44 \pm 0.01	0.23 \pm 0.01	0.42 \pm 0.04	0.87 \pm 0.01
Vanillic acid	0.07 \pm 0.00	0.12 \pm 0.01	0.07 \pm 0.00	0.07 \pm 0.00	0.10 \pm 0.00
Gallic acid	1.15 \pm 0.06	6.33 \pm 0.16	0.55 \pm 0.03	0.95 \pm 0.02	10.79 \pm 0.16
Caffeic acid	1.59 \pm 0.01	6.29 \pm 0.09	0.57 \pm 0.14	0.98 \pm 0.03	9.12 \pm 0.02
Ferulic acid	0.37 \pm 0.02	0.54 \pm 0.02	0.48 \pm 0.03	0.53 \pm 0.01	2.33 \pm 0.01
Sinapic acid	0.02 \pm 0.01	0.04 \pm 0.00	0.03 \pm 0.00	0.05 \pm 0.00	0.28 \pm 0.00
Ellagic acid	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.05 \pm 0.00	0.07 \pm 0.01
<i>Flavonoids</i>					
Apigenin	0.04 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00
Neringenin	0.14 \pm 0.01	0.09 \pm 0.02	0.04 \pm 0.01	0.11 \pm 0.01	0.31 \pm 0.03
Luteolin	0.22 \pm 0.05	0.37 \pm 0.06	0.22 \pm 0.02	0.43 \pm 0.02	1.29 \pm 0.06
Catechin	0.18 \pm 0.03	10.12 \pm 0.78	0.21 \pm 0.01	0.09 \pm 0.01	16.56 \pm 0.06
Epicatechin	0.40 \pm 0.07	1.60 \pm 0.07	0.15 \pm 0.02	0.26 \pm 0.02	2.03 \pm 0.15
Hesperetin	0.02 \pm 0.00	0.03 \pm 0.01	0.02 \pm 0.00	0.02 \pm 0.01	0.27 \pm 0.00
Quercetin	0.15 \pm 0.03	0.12 \pm 0.01	0.14 \pm 0.01	0.12 \pm 0.01	0.15 \pm 0.00
Epigallocatechin	0.17 \pm 0.07	0.27 \pm 0.01	0.09 \pm 0.01	0.18 \pm 0.02	0.22 \pm 0.04
Myricetin	0.20 \pm 0.04	0.15 \pm 0.01	0.24 \pm 0.02	0.26 \pm 0.09	0.50 \pm 0.02
Rutin	1.14 \pm 0.08	1.30 \pm 0.02	0.08 \pm 0.01	0.17 \pm 0.01	0.33 \pm 0.02

Results are represented as mean \pm standard deviation.

Results and Discussion

Total phenol and flavonoid

Among the five plant species *Mallotus tetracoccus* recorded the highest amount of phenolic acids (50.01 $\mu\text{g/mL}$) and flavonoids (25.14 $\mu\text{g/mL}$) followed by *Musa acuminata* (32.32 $\mu\text{g/mL}$ and 18.89 $\mu\text{g/mL}$, respectively). However, lowest amount of phenols (4.81 $\mu\text{g/mL}$) and flavonoids (1.69 $\mu\text{g/mL}$) was found in *Amomum subulatum* (Table 1).

Phenolic acids

A total of eighteen phenolic acids were detected and quantified (Table 2), namely caffeic, chlorogenic, benzoic, gallic, ellagic, gentisic, ferulic, p-coumaric, o-coumaric, protocatechuic, p-hydroxy benzoic, syringic, salicylic, sinapic, vanillic, t-cinnamic, 3-hydroxy benzoic and 2,4-dihydroxy benzoic.

Plant-derived phenolic compounds exert anti-diabetic effects by activating AMPK, inhibiting α -glucosidase and α -amylase leading to enhancement in uptake of glucose and insulin response, and modulating PPARs²¹. They also display anti-cancer activity through apoptosis, autophagy, cell cycle arrest, and telomerase inhibition, with *in-vivo* evidence supporting anti-angiogenic effects²². Furthermore, polyphenols promote vascular health by reducing platelet aggregation and oxidation of LDL, lowering of blood pressure, promoting endothelial functioning, enhancing antioxidant defenses, and suppressing inflammation²³.

Among the five plant species, *Mallotus tetracoccus* reported the highest amount of gallic acid (10.79 $\mu\text{g/mL}$) and caffeic acid (9.12 $\mu\text{g/mL}$) followed by *Musa acuminata* 6.33 $\mu\text{g/mL}$ and 6.29 $\mu\text{g/mL}$, respectively. Gallic acid is naturally present in apple

peel, bark of pomegranate and oak, grapes, sumac and processed products like green tea and wine^{24,25}. Gallic acid and its derivatives are implicated as anti-carcinogenic, anti-microbial, anti-mutagenic, anti-angiogenic and anti-inflammatory agents, to treat various critical illness such as cancer, depression, lipid related diseases, microbial infections, etc.²⁵.

Caffeic acid is one of the principle hydroxycinnamic acid derivative, having various bio-active properties, like antidiabetic, anticancer, antioxidative, antimicrobial, antiviral, antihypertensive, hepatoprotective, etc.²⁶

Flavonoids

Thirteen flavonoids were detected and quantified (Table 2), viz., catechin, apigenin, epigallocatechin, epicatechin, hesperetin, kaempferol, galangin, myricetin, leutolin, naringenin, quercetin, rutin, and umbelliferone.

Highest amount of flavonoids was present in *Mallotus tetracoccus* and *Musa acuminata*; catechin (16.56 µg/mL) preceding epicatechin (2.03 µg/mL) was recorded highest in *Mallotus tetracoccus* followed by *Musa acuminata* i.e., 10.12 µg/mL and 1.60 µg/mL, respectively. Catechin has numerous health benefits viz., anti-cardiovascular, anti-diabetic, anti-obesity, anticancer, anti-infectious, neuroprotective and hepatoprotective effects²⁷. Many researchers reviewed the health beneficial properties of Catechin and its mechanism^{22,28-31}. The essential mechanism resulted in anticarcinogenic effects of Catechin that involves inhibition of cancer cell proliferation, free radical scavenging activity, regulating signalling pathways related to cell cycle²⁸. Catechin exerts thermo genesis, also upregulates the gene involved in lipolysis while down regulating the gene involved in lipogenesis, leading to its antiobesity effects³² as well as cardiovascular disease prevention²⁹. One of the most prominent mechanisms that Catechin performs is ROS scavenging. This mechanism is involved in its various health beneficial properties related to oxidative stress such as anticarcinogenic, anti-diabetic, anti-cardiovascular hepatoprotective and neuroprotective effects²⁷. The other mechanisms underlying antidiabetes role of Catechin include recuperating insulin resistance, regulation of mitochondrial function, ER stress reduction, anti-inflammatory function etc.³⁰. Catechin is found to inhibit phosphorylation of tau protein, reduce amyloid β plaque aggregation, and enhances dopamine level; all these mechanism positively affect against the neurological disorders³¹.

Organic acids

Organic acids from plant extracts display an enormous beneficial property, including hepatoprotection, anti-diabetic, antioxidant, anti-inflammatory, anti-tumoral, and anti-malarial activities³³. They exert these health benefiting effects through the modulation of various cellular and metabolic pathways, such as insulin signaling, apoptosis induction, platelet aggregation, and anti-inflammatory responses³⁴. In the present investigation, eleven organic acids were quantified, viz., fumaric, citric, hydroxycitric, maleic, malonic, malic, lactic, pyruvic, succinic, shikimic, and tartaric (Table 3). The leaf extracts of *Mallotus tetracoccus* is rich in shikimic acid (1217.83 µg/mL) followed by succinic acid (159.22 µg/mL) and malic acid (130.81 µg/mL). *Musa acuminata* contains predominantly Succinic acid (66.09 µg/mL) followed by shikimic acid (61.60 µg/mL). However, *Amomum subulatum* and *Curcuma longa* are rich source of Hydroxycitric acid; 10.68 µg/mL and 24.95 µg/mL, respectively.

Shikimic acid is the major precursor for synthesis of aromatic amino acids, alkaloids, phenolics and phenyl propanoids. It is extensively used in pharmaceutical industries as a chiral building block for the synthesis of Oseltamivir phosphate (Tamiflu), the only drug against swine/ bird flu virus³⁵. Recently, it has also been reported that shikimic acid has novel and multifunctional roles viz., anti-enzymatic activity, dermo-cosmetic composition, and an exfoliating agent for stratum corneum³⁶. At present, shikimic acid is predominantly isolated from the plant sources like, fruits of *Illicium verum*³⁷, however, the leaves of *Mallotus tetracoccus* could be an alternative potential source to extract shikimic acid.

Succinic acid possess the Antioxidant, Anti-diabetic, Anti-obesity properties³⁸ and mallic acid helps in enhancing the muscle antioxidant capacity and possess antimicrobial properties^{34,39}. Hydroxycitric acid has health benefits such as, anti-obesity, anti-carcinogenic and increases serotonin levels in brain⁴⁰.

Water soluble vitamins

A total of seven water soluble B vitamins namely thiamine, niacin, pyridoxine, pantothenic acid, cyanocobalamine, riboflavin and folic acid were present in the leaf sample of different species (Table 3). Niacin is predominantly found in the steamed leaf extracts irrespective of the plant species. *Mallotus tetracoccus* recorded the highest i.e., 31.83 ng/mL followed by *Musa acuminata* (19.23 ng/mL).

(*Mallotus tetracoccus*, *Musa acuminata*, *Curcuma longa*, *Phrynium capitatum*, and *Amomum subulatum*), standardized using Z-scores. The hierarchical clustering reveals distinct metabolic profiles, with *M. tetracoccus* and *M. acuminata* exhibiting relatively higher intensities of phenolic acids and flavonoids such as catechin, epicatechin, and gallic acid suggesting their enrichment in antioxidant-related metabolites. In contrast, *C. longa* and *P. capitatum* clustered more closely, characterized by moderate intensities across diverse metabolites, consistent with their known phytochemical diversity. *A. subulatum* exhibited comparatively lower intensities in most metabolites, except for specific compounds like syringic acid and tannic acid. The separation of species into metabolite-driven clusters underscores their distinct phytochemical fingerprints, reflecting both taxonomic and ecological differences⁴⁵. Such profiling supports the use of metabolomics for species discrimination, functional annotation, and understanding of bioactive compound distribution.

Conclusion

Among the five plants studied, *Mallotus tetracoccus* exhibited the highest levels of phenolics, flavonoids, organic acids, and B-complex vitamins, followed by *Musa acuminata*. However, the high phenolic content of *Mallotus tetracoccus* may impart undesirable bitterness or off-flavor, limiting its suitability for food wrapping. In contrast, *Musa acuminata* leaves, though slightly lower in bioactive compound content, possesses a much milder flavor profile, allowing them to serve as practical wrapping material without altering the sensory attributes of food. In addition to this, *Musa acuminata* is widely cultivated across tropical regions, while its leaves are often treated as agricultural waste, some varieties are even specifically cultivated for their leaves due to their favorable texture and durability. This not only ensures an abundant and low-cost supply but also makes it a sustainable alternative to synthetic plastics. Thus, *Musa acuminata* leaves represent an eco-friendly and health-promoting food wrapping alternative, simultaneously reducing plastic pollution and enriching daily meals with beneficial bioactive compounds.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at [https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_24\(10\)\(2025\)938-946_SupplData.pdf](https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_24(10)(2025)938-946_SupplData.pdf)

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Conflict of Interest

The authors states that no competing financial interests or personal relationships to influence the present study.

Author Contributions

K.S. Shivashankara: Conceptualization, validation, visualization. Mahesh Kumar: Written the manuscript. Siddhartha Singh, Tasso Yatung and Veluru Bhargav: Formulated and prepared the samples. G.A. Geetha⁴, A.N. Loksha: Carried out LC-MS/MS. Veluru Bhargav: Data curation, Formal analysis. Final manuscript version had been read and approved by all the authors.

Data Availability

Supporting data is available in the article and online Supplementary Table S1 to S4.

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