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Effect of Oven-Drying and Lyophilization on Proximate Composition, Ascorbic Acid Content and Screening of Phytochemical Components in *Garcinia xanthochymus* Hook.f. and *Garcinia morella* (Gaertn.Desr) Available in Bodoland Territorial Region (BTR) of Assam, India

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Abstract

The present study aimed to focus on the influence of successive oven drying and lyophilization on proximate composition, ascorbic acid content, and phytochemical screening of *Garcinia xanthochymus* (GX) and *Garcinia morella* (GM). GX extract exhibited high levels of crude fiber (11.45 %), high moisture content (13.92 %), and ash content (3.77 %) compared to GM (crude fiber: 10.25 %, moisture content: 11.45 % and total ash content: 2.78 %). Conversely, GM had higher fat (0.45 %) and crude protein (4.5 %) contents compared to GX (fat: 0.14 %; protein: 4.04 %). Carbohydrates were the most abundant with GX containing 78.13 % and GM 72.23 %. GX also exhibited a higher level of reducing sugars (12.39 %) and ascorbic acid (78.59 mg/100 g) compared to GM (carbohydrate: 3.8 % and ascorbic acid: 72.32 mg/100 g). The variability in nutritional composition was influenced according to species, collection location, and sample source. GC-MS was employed to evaluate phytochemical constituents where GM showed a maximum (30 numbers) in comparison to GX (9 numbers) in terms of alkanes, phenols, fatty acids, terpenes, and steroids.

Keywords: Ascorbic acid; *Garcinia morella; Garcinia xanthochymus*; Proximate analysis.

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1. Introduction

Garcinia is a tropical plant from the Clusiaceae family, found worldwide with over 350 species. It is valued for its ethnomedicinal properties as well as its culinary applications. The value of different species varies based on their regional distribution and availability. For instance, Garcinia indica is primarily found in southern India and is a staple in South Indian cuisine, while Garcinia pedunculata is widely recognized in Northeast India and Bangladesh as a home remedy for stomach ailments. [1].

Garcinia xanthochymus Hook.f., (GX) also known as *Tempwr* in the Bodo language in the Bodoland territorial region (BTR) of Assam, is a tree native to India. It starts fruiting

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from April to August. It grows up to 8-10 meters tall and has dark green leaves. The tree produces sticky yellow sap and yellow fruits about 6-7 cm wide, filled with tart, juicy pulp and two seeds. These fruits are sour and can be used to make jams, preserves, and vinegar. *Garcinia* is also used as a pigment in watercolors and as a dye for fabrics, giving them a yellow color. Traditionally, *Garcinia* fruits have been used by locals as medicine to treat dysentery, constipation, high blood pressure, and headaches. Chemical analyses of various parts of the plant have found compounds like benzophenones, bioflavonoids, flavonoids, triterpenes, xanthones, and lipids. These compounds have been linked to various health benefits such as pain relief, fighting bacteria, antioxidants, and even killing viruses [2-8].

Locally known as *Kuji thekera* in Assamese or *thaika* in the Bodo language in the Bodoland territorial region of Assam and scientifically known as *Garcinia morella* (Gaertn. Desr.), (GM) is another fruit-bearing tree related to mangosteen. It starts to blossom during April and starts fruiting in September. It grows up to 12 meters tall and is found in India, Indochina, and Sri Lanka. The tree has simple leaves and dark brown bark that produces yellow resin. Its berries are about 3 cm wide, contain four seeds, and are used as a dessert fruit. A survey of literature revealed its potential as a lipid-lowering agent by the ability to lower cholesterol, triglycerides, and Low-Density Lipoprotein Cholesterol (LDLc). The fat extracted from the seeds is used in cooking and confectionery. The resin is used as a yellow dye, illuminant, and in varnishes and watercolors. It is collected by slicing the bark and scraping off the dried resin. Indian gamboge contains various secondary metabolites like xanthones, flavonoids, benzophenones, phenolic acids, organic acids, triterpenoids, and fatty acids, which have different uses and benefits [3-5,9-12].

2.1. *Materials*

The fruits of GX and GM are sourced from the local markets and known places from the four districts of the Bodoland Territorial Region of Assam, India. Freshly harvested fruits were brought to the laboratory for processing.

2.2. *Methods*

Fruits gathered were sorted, cleaned under the running tap water, and dried with a paper towel. After cleaning, the fruits were sliced and the seeds were removed. The pericarp of the fruits was cut into thin slices and kept for oven drying at ≤ 60 °C to replicate the sun drying process of the traditional method followed by the locals. The samples were oven-dried till constant weight was obtained. Following this, the dried samples were ground into fine powder using the kitchen blender and stored for future use. For the extraction process, the dried pericarp samples were subjected to cold maceration in methanol for three days with occasional shaking. The resulting extract was filtered through Whatman filter paper 1 and concentrated into the dried form using a rotary vacuum evaporator (Equitro Xtemp, Roteva model) and lyophilized to remove any moisture content. The resulting sample was stored under 20 °C for further use [13].

2.2.1. *Proximate analysis*

The determination of proximate analysis consists of moisture content, ash content, crude fat, and total carbohydrates. It was determined using the AOAC method [14]. Crude fiber was determined by following the Indian standard method 10226 [15]. The Indian Standard 7219 method was employed to find out the protein content [16].

2.2.2. *Determination of ascorbic acid*

The ascorbic acid in fruit samples was determined by extracting them with a 3 % oxalic acid solution and then titrating them with 2,6-dichlorophenolindophenol [17].

2.2.3. *Determination of reducing sugar*

The dinitrosalicylic acid (DNS) method was followed to measure the reduced sugar content. When reducing sugars were present, DNS was reduced to 3-amino-5-nitrosalicylic acid, observing an orange-red color that was measured at 510 nm [18].

2.2.4. *Screening of bioactive compounds*

The methanolic extract of *Garcinia* varieties of both GM and GX was screened for the presence of phytochemical compounds using Gas Chromatography-Mass Spectrometry (GC-MS). The method was followed with minor modifications outlined by Casuga *et al*. [19]. Types of equipment used were Scion 436-GC Bruker, equipped with a Retention time Rtx-5MS column (5 % Diphenyl / 95 % Dimethylpolysiloxane), measuring 30 m × 0.25 mm ID × 0.25 µm df. The software used for Mass spectrometric detection was Xcaliber software. Pure helium gas (99.995 %) was used as carrier gas at a flow rate of 1 mL/min. The sample was injected after diluting with the appropriate solvent measured at 1 µL of a 1 % solution of the sample extracts. The oven temperature was set at 110 °C held for 3.50 min, then ramped up to 200 °C at a rate of 10 °C/min with no hold, subsequently increased to 280 °C at a rate of 5 °C/min and maintained for 12 min. The injector temperature was set at 280 °C. The total runtime for GC was set to 40.50 min. The inlet line temperature was set to 290 °C, and the source temperature was maintained at 250 °C. Electron energy was set at 70 eV, and mass scanning (m/z) ranged from 50 to 500 Atomic Mass Units (amu). A solvent delay of 0 to 3.5 min was implemented. The total running time for MS was 40.50 minutes. The National Institute of Standards and Technology Version-2011 library was used to determine the analysis. The proportion of chemical compounds found in the extract was calculated as a percentage using the peak area shown in the chromatogram. All assays were conducted in triplicate, and the results were expressed as the Standard Error of the Mean (SEM).

3. Results and Discussion

3.1. *Proximate analysis*

It provides essential information on food components which helps to identify, classify, and determine the precise nutritional content of the nutrients. Table 1 presents the proximate composition of GX and GM fruits which revealed high levels of Crude Fiber. GX has a higher content of crude fiber with 11.45 ± 0.02 % when compared to GM i.e. 10.25 ± 0.03 %. Moisture content was lower in GM 12.43 ± 0.21 % compared to GX 13.92 ± 0.23 %. Low moisture content means less microbial load hence it can be stored longer. Total Ash content was significantly higher in GX 3.77 ± 0.04 % when compared to GM 2.78 ± 0.02 %. Fat content was higher for GM at 0.45 ± 0.01 % compared to GX at 0.14 ± 0.005 %. Similarly, crude protein was also found to be present higher in GM i.e. 4.5 ± 0.05 % compared to GX which is 4.04 ± 0.01 %. Compared to an earlier study by Janhavi *et. al*. [6] on GX revealed higher content of crude fiber on different fruit parts where the content was highest in peel (16.31 ± 0.35 %) compared to our GX sample which recorded 11.45 ± 0.02 %. This difference may be due to the locations of species and source of collection. Similarly, the fat content also followed the same trend as carbohydrate content. Carbohydrate was found to be the most abundant constituent in both the species where GX has 78.13 ± 2.05 % followed by GM i.e. 72.23 ± 2.23 %. Proximate analysis is widely used in food research and education to study the nutritional properties of different foods, investigate dietary patterns and their impact on health, and develop evidence-based dietary recommendations. It furnishes valuable insights into fundamental nutritional elements such as moisture content, ash, protein, fat, fiber, and carbohydrates within food samples. Understanding these components is crucial for assessing the nutritional value of foods and their potential health benefits.

The proximate analysis revealed that lyophilized and oven-dried fruits are rich in nutritional properties. Both GM and GX had a high content of crude fiber, protein, and fat. They also showed an abundance of carbohydrate content. Furthermore, the composition of reducing sugars results revealed that GX (12.39 ± 0.11 %) had a high level of reducing sugars compared to GM (3.8 ± 0.03 %). The reducing sugar test specifically helps quantify the amounts of sugars present in a sample, which contributes to the overall carbohydrate content. Therefore, it is an important component of proximate analysis for understanding the nutritional profile of a food sample. Within the proximate analysis, the reducing sugar test is frequently integrated, serving as a method to ascertain the basic nutritional composition of food. This analysis quantifies the presence of reducing sugars within a sample. These sugars, capable of reducing specific compounds like copper ions through electron donation, are commonly found in carbohydrates such as glucose, fructose, and maltose. However, GX showed a relatively higher amount of reduced sugar compared to GM. Another study by Sharma *et al*. [20] revealed less crude fiber content (2.73 ± 0.62 %) compared to our sample (11.45 ± 0.02 %). Therefore, it is an important component of proximate analysis for understanding the nutritional profile of a food sample. Similar

findings were observed in studies of GX by Murmu *et al*. [21]. A study conducted on GM also showed that the seed extracts had a high amount of carbohydrates and were rich in oil content (38.08 g/100 g) but had low crude protein content [22].

Table 1. Proximate composition, reducing sugar and ascorbic acid determination of *G. xanthochymus* and *G. morella.*

Test Parameters	GX (g/100 g)	GM (g/100 g)
Moisture	13.92 ± 0.23	12.43 ± 0.21
Total Ash	3.77 ± 0.04	2.78 ± 0.02
Crude Fiber	11.45 ± 0.02	10.25 ± 0.03
Crude Fat	0.14 ± 0.005	0.45 ± 0.01
Crude Protein	4.04 ± 0.01	4.5 ± 0.05
Carbohydrate	78.13 ± 2.05	72.23 ± 2.23
Reducing Sugar	12.39 ± 0.11	3.8 ± 0.03
Ascorbic acid	78.59 ± 2.57 (mg/100 g)	72.32 ± 3.46 (mg/100 g)

Results were expressed as mean ± standard error mean (SEM), and differences between samples were determined by the multiple range test. *P* values at < 0.05 were regarded as significant.

3.2. *Determination of ascorbic acid*

It is also known as vitamin C, which is a specific nutrient rather than a basic nutritional component, and its analysis is usually performed separately from proximate analysis. However, it is often included in dietary analyses to provide a more comprehensive understanding of the nutritional profile of a food sample. The ascorbic acid content was determined and was found to be higher in GX (78.59 ± 2.57 mg/100 g) than in GM (72.32 ± 3.46 mg/100 g) (Table 1). It is an essential water-soluble vitamin known to have antioxidant properties, and its analysis is important in various fields including food science, nutrition, pharmaceuticals, and biological research. Fig. 1 shows graphical visualization of proximate compositions, reducing sugar, and ascorbic acid analysis of GX and GM.

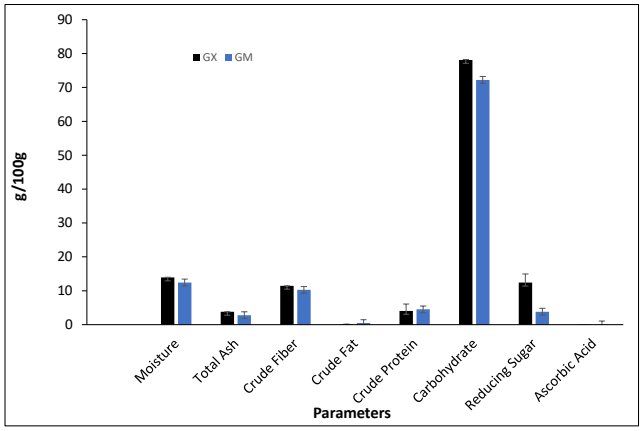
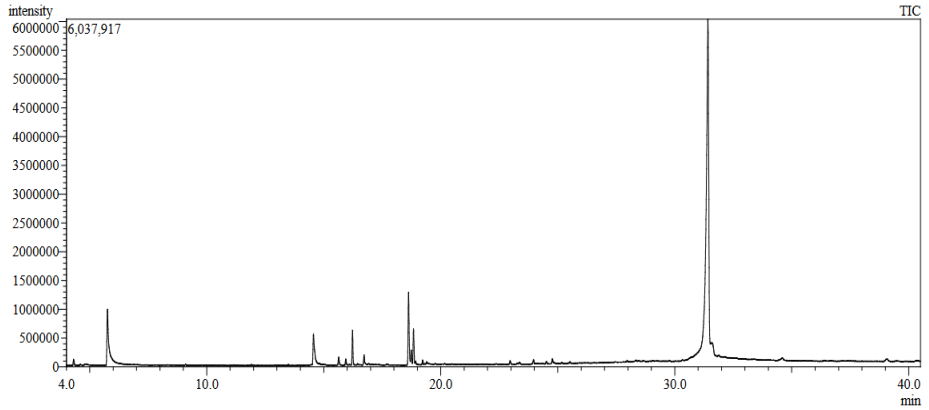


Fig. 1. Proximate compositions, reducing sugar, and ascorbic acid analysis of *Garcinia xanthochymus* (GX) and *Garcinia morella* (GM).

3.3. *Determination of bioactive compounds*

It was applied to qualitatively assess the various phytochemical compounds within crude extracts of *G. xanthochymus* and *G. morella*. Table 2 presents the phytochemical compounds screened from the methanol extracts of *G. xanthochymus* whereas Table 3 presents the phytochemical compounds screened from the methanol extracts of *G. morella*. Fig. 2 illustrates the chromatogram graphs of *G. morella* and *G. xanthochymus.*

A. *Garcinia morella*



B. *Garcinia xanthochymus*

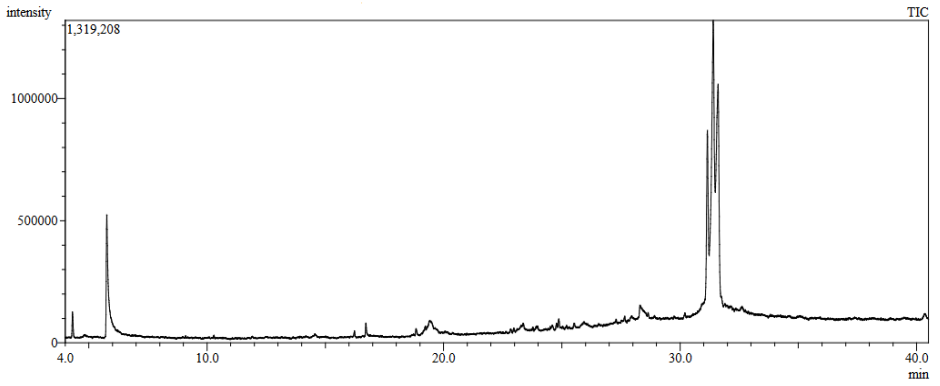


Fig. 2. GC-MS analysis of A) *Garcinia morella* and B) *Garcinia xanthochymus* sourced from BTR, Assam, India.

Table 2. Bioactive compounds present in *Garcinia xanthochymus*.

No	RT	Name of the compound	Category	Peak Area %
1	4.312	Undecane	Alkane	1.13
2	5.762	Catechol	Phenol	10.9

3	16.234	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty acid methyl ester	0.2
4	16.716	n-Hexadecanoic acid	Fatty acid	0.56
5	24.875	1,2-15,16-Diepoxyhexadecane	Epoxy compound	0.37
6	31.151	.alpha.-Damascone	Ketone	13.82
7	31.399	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	Terpene	41.01
8	31.606	Bicyclo[4.2.0]oct-2-ene, 3,7-dimethyl-7-(4-methyl-3-pentenyl)-8-(2,6,10-trimethyl-1,5,9-undecatrienyl)-, [1.alpha.,6.alpha.,7.b	Terpene	31.59
9	31.765	Hexan-1,5-dione, 3-ethyl-3,4-dimethyl-1-[10-methyldecalin-1,4-dien-3-one-9-yl]-	Ketone	0.41

Table 3. Bioactive compounds present in *Garcinia morella*.

No	RT	Name of the compound	Category	Peak area %
1	4.312	Undecane	Alkane	0.39
2	5.757	Catechol	Phenol	7.83
3	11.92	Hexadecane	Alkane	0.06
4	13.492	Tridecanoic acid, methyl ester	Fatty acid methyl ester	0.04
5	14.562	1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	Terpene	3.66
6	15.638	1-Hexadecanol	Alcohol	0.57
7	15.942	9-Octadecenoic acid (Z)-, methyl ester	Fatty acid methyl ester	0.4
8	16.232	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty acid methyl ester	2.2
9	16.724	n-Hexadecanoic acid	Fatty acid	0.68
10	18.623	1-Hexadecanol	Alcohol	5.89
11	18.739	9,12-Octadecadienoic acid, methyl ester, (E, E)-	Fatty acid methyl ester	1.07
12	18.839	9-Octadecenoic acid (Z)-, methyl ester	Fatty acid methyl ester	2.5
13	18.921	9-Octadecenoic acid (Z)-, methyl ester	Fatty acid methyl ester	0.24
14	19.225	Methyl stearate	Fatty acid methyl ester	0.29
15	19.403	Oleic Acid	Fatty acid	0.15
16	22.968	trans-Farnesol	Terpene alcohol	0.23
17	23.367	4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one	Ketone	0.13
18	23.967	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	Terpene	0.28
19	24.512	trans-Farnesol	Terpene alcohol	0.16
20	24.77	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	Terpene alcohol	0.35
21	25.519	Decanal	Aldehyde	0.08

22	30.695	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E, E, E)-	Terpene alcohol ester	0.15
23	30.79	3.beta.-Myristoyl-28-(trifluoroacetyl)olean-12-ene	Triterpenoid	0.31
24	30.89	Pregnane-3,11,20-trione, (5.beta.)-	Steroid	0.39
25	30.96	Octadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	Fatty acid derivative	0.61
26	31.418	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	Terpene	67.64
27	31.572	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	Terpene	3.23
28	31.775	1,1'-Bicyclohexyl, 4-propoxy-4'-propyl-	Bicyclic compound	0.09
29	34.585	Squalene	Triterpene	0.11
30	39.069	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	Terpene	0.3

GC-MS is a powerful analytical technique used to identify and quantify the components of complex mixtures of chemicals. Phytochemicals play a crucial role as primary constituents responsible for the bioactive potential of plants. Consequently, it has become increasingly vital to ascertain the phytochemical makeup of plants. The production of diverse phytochemicals, facilitated by the development of various physiological mechanisms, stands out as one of the most successful adaptations of plants. Through these mechanisms, plants effectively safeguard themselves from both biotic and abiotic stresses. The health benefits associated with numerous medicinal plants or fruits are credited to their content of phytochemicals. Conditions like diabetes, obesity, cancer, and cardiovascular diseases often arise from persistent low-grade inflammation and oxidative stress. Hence, many studies focus on exploring the anti-inflammatory and antioxidative properties of phytochemicals. The aim is to develop these compounds into nutraceuticals for preventing metabolic disorders. Screening of phytochemical compounds in GX and GM revealed various organic compounds that were identified to have health benefits and other commercial importance. GX revealed 9 organic compounds whereas GM showed 30 organic compounds. These compounds are categorized as alkanes, phenols, fatty acid methyl esters, terpenes, alcohols, fatty acids, ketone, aldehyde, triterpenoid, steroids, bicyclic compounds, and Triterpene. Among these compounds, several have been studied for potential health benefits. Catechol is a phenol compound known for its antioxidant properties, which may contribute to various health benefits, including protecting cells from oxidative damage. Certain terpenes, such as trans-Farnesol and Squalene, have been investigated for their potential health-promoting effects. For instance, Squalene is found in shark liver oil and some plant oils and has been studied for its antioxidant and immune-boosting properties. Oleic acid, a monounsaturated omega-9 fatty acid found in olive oil, has various health benefits, including promoting heart health and reducing inflammation. Additionally, octadecanoic acid, also known as stearic acid, is a saturated fatty acid found in some foods like cocoa butter and has been shown to have neutral effects on cholesterol

levels. Compounds like 3.beta.-myristoyl-28-(trifluoroacetyl) olean-12-ene (a triterpenoid) and Pregnane-3, 11, 20-trione (a steroid) have been studied for their potential pharmacological properties, although their specific health benefits may vary depending on their structure and activity. Similarly, studies conducted by Pandey *et al*. [10] showed the presence of xanthones, benzophenones, flavonoids, organic acids, phenolic acids, and triterpenes in the leaf of GM. Benzophenone was also derived from the fruit of GM by Choudhury *et al.* [11]. Likewise, similar findings were interpreted by studies on GM by Bheemaiah *et al*. [23]. Various phytochemical constituents are extracted from GX, and present in diverse plant parts such as the bark, fruit, leaves, and others. Similarly, the fruit of GX contains phytochemicals that have been isolated and extracted, including flavonoids, benzophenones, and xanthones. Xanthones, such as atroviridin, constitute the majority of the phytochemicals that can be extracted and isolated from the bark. Additionally, a flavonoid compound, morelloflavone, has been identified in the bark extract of this species. Furthermore, a study has also discovered a novel compound from the depsidone group, termed garciniadepsidone [21-26]. A review by Mustafa *et al*. on *Garcinia celebica* indigenous to Southeast Asian countries documented approximately 100 phytochemicals, encompassing various compound classes such as triterpenoids, flavonoids, benzophenones, xanthones, depsidones, and sterols. Among these, triterpenoids and xanthones were the most abundant. Extracts and isolated compounds from these classes have demonstrated a range of biological activities, including antibacterial, antiparasitic, hepatoprotective, antioxidant, antidiabetic, antituberculosis, antiplatelet aggregation, anti-neuraminidase, and cholinesterase inhibitory effects [27]. Another documentation on the crude fruit extract of Garcinia gummi-gutta has demonstrated anti-inflammatory, anticancer, anthelmintic, antimicrobial, and antioxidant properties in both in vivo and in vitro studies. The fruit rind holds significant medicinal value and is widely used in Ayurveda and traditional medicine for treating various ailments**.** G. gummi-gutta contains diverse secondary metabolites, including organic acids such as hydroxycitric acid (HCA), flavonoids, terpenes, polysaccharides, and polyisoprenylated benzophenones like garcinol, xanthochymol, guttiferone, as well as benzophenones, xanthones, biflavonoids, alkaloids, tannins, phenols, and saponins [28]. While these compounds have been investigated for potential health benefits, individual responses to these compounds may vary, and further clinical research is needed to gain in-depth knowledge of how these compounds interact within our bodies.

4. Conclusion

Garcinia, being an underutilized fruit has already found its utility by local consumers of Bodoland territorial region (BTR), Assam. However, it needs to be explored in terms of its nutritional value and functional bioactive constituents which provide potential health benefits. The proximate analysis of oven-dried and lyophilized *Garcinia* species highlights their potential nutritional properties in terms of high levels of crude fiber, protein, fat, and carbohydrates, reducing sugar and ascorbic acid contents. Comparisons with previous studies underscore the variability in nutritional composition and parameters varied in the

present investigation as compared with previous studies due to several factors for example nature of species, collection area, and sample source. GC-MS is an important tool to identify various bioactive or phytochemical constituents in both these two processed *Garcinia* species and provides significant health benefits. Further investigation is warranted to elucidate their mechanisms of action, individual variability, and consumption patterns. Consumption of these compounds as part of a balanced diet is recommended, emphasizing the importance of holistic dietary patterns over isolated supplementation.

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