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Optimising the Eco-Friendly Extraction of Carotenoids From Gac Fruit: A Sustainable Solution for Natural Red Food Colourants

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ABSTRACT

Plant-based red food colours encounter significant challenges regarding pH sensitivity, off-flavours, processing instability and reduced vibrancy compared to synthetic dyes. These issues, along with high costs and inconsistent supply, highlight the urgent need for alternative sources, stabilisation technologies and scalable production. Gac fruit (*Momordica cochinchinensis* Spreng) is one of the rich natural sources of red-hued carotenoids. This study was conducted with an objective to come up with an efficient, low-cost and eco-friendly method for carotenoid pigment extraction from gac fruit with the perspective of food application. Among the different methods, the extraction method using ethyl lactate showed the highest concentrations of total carotenoids (2069.83 ± 4.38 μg/g), β-carotene (689.43 ± 1.04 μg/g), lycopene (1052.31 ± 0.87 μg/g) and lutein (216.96 ± 0.22 μg/g). The pigment concentrate obtained using ethyl lactate solvent exhibited superior instrumental colour values: L* (60.44 ± 0.12), a* (61.35 ± 0.20), b* (43.24 ± 0.10), chroma (65.62 ± 0.10) and hue angle (36.64 ± 0.18); highest total phenolics (211.57 ± 0.13 mg gallic acid equivalent (GAE)/100 g); and antioxidant properties analysed using DPPH (2,2-diphenyl-1-picrylhydrazyl) (0.59 ± 0.005 μL/mL), FRAP (ferric reducing antioxidant power) (0.63 ± 0.004 μL/mL) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (0.58 ± 0.005 μL/mL) assays. The LC-MS/MS analysis of gac fruit carotenoid pigment concentrate indicated the presence of various health-beneficial bioactive compounds such as flavonoids, phenolic compounds, terpenoids, diterpenoids, triterpenoids, alkaloids, glycosides and saponins, benzopyrans and phenylpropanoids, macrolides, fatty acid derivatives and glycosylmonoacylglycerols. These results demonstrate the potential of gac fruit as a valuable natural source of red pigment for food applications and show that ethyl lactate is an effective and eco-friendly solvent for optimising carotenoid recovery.

1 | Introduction

Gac fruit (*Momordica cochinchinensis* Spreng.), a member of the Cucurbitaceae family, is indigenous to Southeast Asia, where it has a rich tradition of use in both dietary and medicinal practices. Revered as the 'Fruit of Heaven' in Vietnam, it is valued for its vivid red colour, which is attributed to its high carotenoid content, particularly in the fruit skin and seed arils (Vuong et al. 2006). In addition to carotenoids, gac fruit contains other bioactive compounds such as phenolics and flavonoids, making it a potent source of natural antioxidants (Chuyen et al. 2017).

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The vibrant red, orange and yellow hues of various fruits, vegetables and flowers are due to carotenoids, which are lipid-soluble pigments that are widely present in the plant kingdom (Mortensen 2006). Carotenoids, such as β-carotene, lutein and lycopene, are not only colourants but also critical for human health, acting as antioxidants and providing provitamin A activity. With their potential for use as natural food colourants, antioxidants and health-promoting agents, carotenoids have garnered significant attention for food industry applications, particularly as natural alternatives to synthetic colours. In the food industry, there is an increasing demand for natural colour sources owing to consumer concerns regarding synthetic additives. Plant-based red food colours encounter significant challenges regarding pH sensitivity, off-flavours, processing instability and reduced vibrancy compared to synthetic dyes. Gac fruit, with its rich and stable red pigment derived from carotenoids, can be a promising alternative for colouring food products. Natural red colourants from gac offer multiple benefits; viz., they are not only safer but also provide additional health benefits through antioxidant activity. However, the potential of gac fruit as a commercial source of natural red pigment remains underutilised because of the lack of economical and eco-friendly extraction methods that can maximise the recovery of its valuable carotenoids.

Traditional solvent extraction techniques have been employed for the recovery of bioactive compounds in food and pharmaceutical applications. However, these methods often involve the use of organic solvents, which may leave residual contaminants and pose safety concerns (Arnold and Gramza-Michalowska 2023). Various studies have explored different solvents to maximise carotenoid extraction efficiency from plant materials, with ethanol being frequently used because of its effectiveness, relative safety and food-grade status. The ability of ethanol to penetrate plant cell walls makes it a widely accepted solvent, particularly when combined with other solvents such as ethyl acetate, to enhance the extraction of both polar and nonpolar compounds. For instance, Saadedin et al. (2017) tested solvent mixtures, such as ethanol/ethyl acetate and hexane/acetone, for extracting lycopene and β-carotene from gac fruit, demonstrating improved recovery compared to single solvents. Similarly, Pandya et al. (2017) optimised lycopene extraction from tomato pomace using acetone and ethyl acetate and achieved high lycopene yields under specific conditions. Although such combinations can boost extraction efficiency, they often fall short of green chemistry standards owing to the potential environmental and health hazards associated with the disposal of hazardous solvents. Microwave-assisted extraction (MAE) has been proposed as an alternative method to address these concerns, enhancing the extraction efficiency by applying microwave energy to rapidly heat the solvent and plant matrix, which disrupts cell walls and facilitates the release of bioactive compounds. This technique can reduce extraction time, lower solvent requirements and improve yield, especially when used with safer solvents such as ethanol. However, MAE can sometimes degrade heat-sensitive compounds and may require specialised equipment, which adds to operational complexity. Thus, although advancements in extraction methods and solvent combinations have improved carotenoid yields, there remains a need for scalable, sustainable and safe techniques that align with green chemistry principles, making natural bioactives more accessible for food and pharmaceutical applications.

One promising alternative is ethyl lactate, a 'green' solvent derived from carbohydrate fermentation, which is nontoxic and suitable for food use. The unique amphiphilic properties of ethyl lactate allow it to solubilise both hydrophilic and hydrophobic compounds, making it effective for extracting a range of phytochemicals, including carotenoids and polyphenols (Pereira et al. 2011). Additionally, ethyl lactate is safe for food applications and meets the regulatory standards set by the European Food Safety Authority (2013) and the U.S. FDA (2014). Given its environmental benefits and effectiveness in solubilising bioactive compounds, ethyl lactate is an optimal alternative for carotenoid extraction from gac fruit, aligning with the principles of sustainable extraction technology. The aim of this study was to come up with an efficient, low-cost and sustainable extraction technique with the objective of maximising the recovery of carotenoid pigment with the perspective of food application. Furthermore, profiling of bioactive compounds through LC-MS/MS analysis was conducted to enable a better understanding of the potential phytochemicals present in gac fruit.

2 | Materials and Methods

2.1 | Sample Preparation: Collection and Preparation of Gac Fruit Seed Aril

The maturity of gac fruit was indicated by the bright red colour completely spreading over the pericarp. Subsequently, the extracted aril alone was dried in a cabinet dryer at 40 ± 2°C until the moisture content became constant. The dried aril was then ground in a commercial blender and sieved through an 80-mesh sieve. Later, the aril powder was packed in polyethylene-laminated aluminium pouches and stored in a freezer until pigment extraction.

2.2 | Extraction of Carotenoid Pigment

Carotenoid pigment extraction from gac fruit seed aril was carried out using the solid-liquid extraction method with different solvents, which included the following: T_1—ethanol, T_2—ethyl lactate, T_3—ethanol:ethyl lactate (1:1 ratio), T_4—microwave-assisted extraction with ethanol and T_5—microwave-assisted extraction with ethanol:ethyl lactate (1:1 ratio). The sample was heated in a solvent at a temperature of 45°C for 45 min. The ratio of plant sample to solvent was 1:20 (w/v), that is, 0.05 g/mL. In the case of MAE, the sample was mixed with the respective solvents, and the tube containing the suspension was irradiated in the microwave device at 300 W for 120 s with the temperature maintained between 45°C and 50°C. The extract was filtered through filter paper. The filtrate was collected and evaporated using a rotary vacuum evaporator (Heidolph rotary evaporator, Germany) at 60°C and 50 mbar. The concentrated filtrates were

kept in glass vials, packed in polyethylene-laminated aluminium pouches and stored under refrigerated (4°C–7°C) conditions until analysis (Figure 1a).

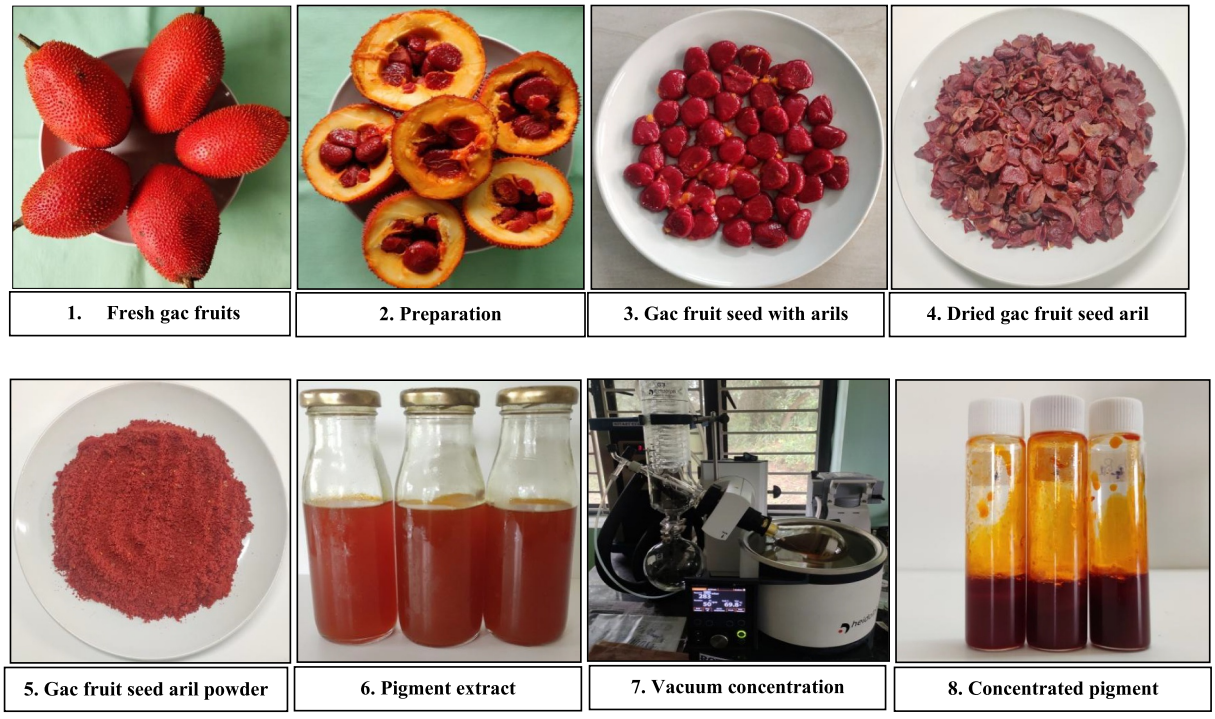
2.3 | Liquid Chromatography-Mass Spectrophotometry (LC-MS/MS)

Analysis was performed using a Q-TOF mass spectrophotometer. The compounds were identified using a high-resolution Orbitrap mass spectrometer at SAIF, IIT Bombay. The data acquisition software used was 6200 series TOF/6500 series version Q-TOF B.05.01 (B5125.3). Chromatography was performed on an Eclipse Plus C18 150 × 2.1 mm-5 micron column. The mobile phase consisted of 0.1% formic acid in Milli-Q water (A) and methanol (B) at a flow rate of 0.3 mL/minutes.

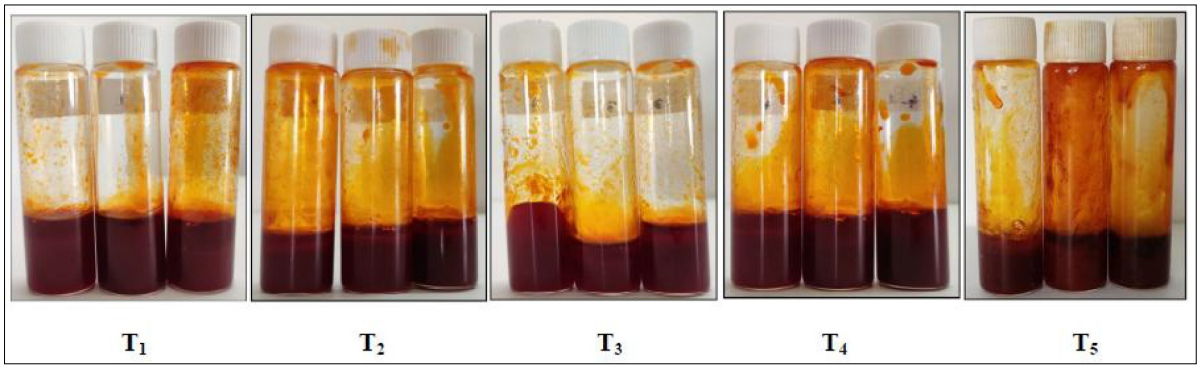
2.4 | Recovery percentage of the Carotenoid Pigment Concentrate (%)

The recovery percentage of the carotenoid pigment concentrate was determined using Equation (1) (Gonfa et al. 2020):

$$\text{Recovery percentage of the carotenoid pigment concentrate (\%)} = \frac{\text{Weight of pigment concentrate (g)}}{\text{Weight of powdered sample (g)}} \text{x } 100 \quad (1)$$



(a)



(b)

FIGURE 1 | (a) Steps followed for the extraction of carotenoid pigment from gac fruit seed aril. (b) Impact of extraction techniques on carotenoid profile of gac seed aril pigment concentrates [T_1—ethanol; T_2—ethyl lactate; T_3—ethanol and ethyl lactate (1:1 ratio); T_4—microwave-assisted extraction with ethanol; T_5—microwave-assisted extraction with ethanol and ethyl lactate (1:1 ratio)].

2.5 | Total Carotenoid Content (μg/g)

A known quantity of the sample was dissolved in 2 mL of n-hexane and mixed thoroughly for 2 min. The absorbance was measured using a spectrophotometer (UV–visible 1800 spectrophotometer, Shimadzu, Kyoto, Japan) at 445 nm. Finally, the absorbance of each sample was compared with a standard curve ($R^2 = 0.99$) to calculate the concentration of carotenoids in μg/g of pigment extract (Natnoi and Pirak 2019).

2.6 | β-carotene and Lycopene Content (μg/g)

The β-carotene and lycopene content of the pigment concentrate from gac fruit was determined using the method described by Vieira et al. (2020). A known quantity of sample was placed in a test tube. A mixture of acetone and hexane at a ratio of 4:6 (v/v) was added and mixed well with the sample. The mixture was centrifuged at 15,000 rpm for 1 min. Absorbance (A) values at 453 and 505 nm were recorded to determine β-carotene and lycopene content in each sample and were calculated using Equations (2) and (3), respectively:

$$C_{\beta-carotene} = 4.624 * A_{453} - 3.091 * A_{505} \quad (2)$$

where $C_{\beta\text{-carotene}}$ is the concentration of β-carotene expressed in μg/g and A_{453} and A_{505} represent the absorbance at 453 and 505 nm, respectively.

$$C_{Lycopene} = 3.956 * A_{453} - 0.806 * A_{505} \quad (3)$$

wherein $C_{Lycopene}$ is the concentration of lycopene expressed in μg/g; A_{453} and A_{505} represent the absorbance at 453 and 505 nm, respectively.

2.7 | Lutein Content (μg/g)

A known quantity of sample (0.01 g) was mixed with 10 mL of absolute ethanol. The solution was filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 10,000 rpm for 10 min. The aqueous phase was collected, and absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 445 nm (Kamalambigeswari and Rebecca 2016) and calculated using Equation (4):

$$\text{Concentration of lutein } (\mu g/g) = \frac{A \text{ x } V(ml) \text{ x dilution factor}}{E^{1\%}cm \text{ x } W\ (g)} \text{x } 100 \quad (4)$$

where A = absorbance, V = volume (mL), $E^{1\%}$ cm = extinction coefficient of solvent (2550 in ethanol at 445 nm) and W = weight of the sample (g).

2.8 | Colour Properties

The colour of the samples in terms of instrumental colour values (L*, a*, b*, chroma and hue angle) was measured by reflectance measurements using a Minolta CM-3600d (Konica Minolta Sensing Inc., Osaka, Japan) with JAYPAK 4808 software (quality control system, version 1.2) based on the CIELAB colour space. A D_{65} lamp was used as a reference light source. The colorimeter measured the lightness (L*) value and the coordinates a* and b*. Lightness (L*) values of 100 and 0 represent absolute white and absolute black, respectively, whereas positive and negative a* (+a* and −a*) values denote the direction of redness and greenness, respectively. The values of positive and negative b* (+b* and −b*) are in the direction of the vector for yellowness and blueness, respectively. The chroma (C*) value was a measure of intensity (or saturation), whereas the hue angle (h°) was explained and characterised by the colour wheel, wherein the angles 0°, 30°, 60°, 90°, 120°, 150°, 180° (or −180°), 210° (−150°), 240° (or −120°), 270° (−90°), 300° (−60°) and 330° (−30°) were represented by red, orange, yellow, lime, green, turquoise, cyan, cobalt, blue, violet, magenta and crimson colours, respectively (Azima et al. 2017).

2.9 | Total Phenolics (mg Gallic Acid Equivalent (GAE)/100 g)

Total phenolics were estimated using Folin–Ciocalteu reagent, as described by Lakshan et al. (2019) with slight modifications. A known quantity of the sample was diluted with distilled water and mixed with Folin–Ciocalteu reagent (0.5 mL), followed by the addition of sodium carbonate solution (1.5 mL) to the mixture. The mixture was incubated at 70°C for 10 min. The absorbance was measured at 765 nm using a spectrophotometer (UV–visible 1800 spectrophotometer, Shimadzu, Kyoto, Japan). The total phenolic content was quantified using a calibration curve prepared using a gallic acid standard. The results are expressed as mg GAE per 100 g sample.

2.10 | Antioxidant Activity

2.10.1 | DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay (μL/mL)

The DPPH radical scavenging activity of the samples was determined based on the method developed by Chaovanalikit et al. (2012), with slight modifications. A known quantity of sample was added to methanol. To this mixture, 0.2 mL of DPPH radical solution was added, and the resulting mixture was incubated at 25 ± 2°C for 30 min. After incubation, the absorbance was recorded at 517 nm. The radical scavenging percentage was calculated using Equation (5):

$$\text{Per cent inhibition} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \text{x } 100 \quad (5)$$

The sample concentration providing 50% reduction in colour (IC_{50}) was calculated from the graph of the per cent inhibition versus the concentration. Gallic acid was used as the standard.

2.10.2 | FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP assay determines reducing power capacity (Benzie and Strain 1996). The FRAP reagent was prepared by adding 10 parts of 300 mM acetate buffer (pH 3.6), 1 part of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) prepared in 40 mM hydrochloric acid (HCl) and 1 part of 20 mM ferric chloride ($FeCl_3$). The mixture was diluted to one third with methanol and prewarmed to 37°C. This reagent (3 mL) was mixed with 0.1 mL diluted test samples. The mixture was shaken and incubated in the dark at 37°C for 20 min, and the absorbance was measured at 593 nm. Ascorbic acid was used as the standard. The sample concentration

providing 50% reduction in colour (IC_{50}) was calculated from the graph of the per cent inhibition versus the concentration.

2.10.3 | *ABTS [2,2′-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid)] Assay (μL/mL)*

ABTS radical scavenging activity was determined according to Brand-Williams et al. (1995) with some modifications. To prepare the ABTS stock solution, potassium persulfate (2.45 mM) and ABTS (7 mM) were mixed at an equal ratio (1:1) and left in the dark for at least 16 h. The working solution was prepared by combining the ABTS stock solution with methanol before use to obtain an absorbance of 0.706 ± 0.01. After allowing the sample to react with the ABTS solution for 7 min, the absorbance was measured at 734 nm using a spectrophotometer. The appropriate solvent blank was used. The ABTS scavenging capacity of the pigment concentrate was compared to that of ascorbic acid, and the percentage inhibition was calculated using Equation (6). The sample concentration providing a 50% reduction in colour (IC_{50}) was calculated from the graph of the per cent inhibition versus the concentration.

$$\text{Scavenging activity} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \text{x } 100 \quad (6)$$

where absorbance (control) is the absorbance of the ABTS radical in methanol and absorbance (sample) is the absorbance of the ABTS radical solution mixed with the sample/standard.

2.11 | Statistical Analysis

The experiment was repeated four times, and the results were expressed as mean values with standard deviation (± SD). One-way analysis of variance (ANOVA) was performed to determine significant group differences ($p \leq 0.05$) between means. Duncan's multiple range test (DMRT) was used to compare the mean values.

3 | Results and Discussion

3.1 | LC-MS/MS Analysis

The parameters used to identify a chemical through LC-MS/MS analysis included a retention time difference of ± 0.15 min and a target compound score of more than 50 out of 100. In practice, these criteria were broad, as most compounds exhibited average scores of 87/100 and retention time differences of ≤ 0.05 min. By applying these parameters, compounds with the same molecular mass and target chemical formula were no longer falsely identified as positive compounds. The LC-MS/MS analysis of gac fruit carotenoid pigment extract revealed the presence of numerous bioactive compounds. Table 1 presents the results for the extracted and examined chemicals.

3.2 | Recovery Percentage of Carotenoid Pigment Concentrate

The amount of carotenoids extracted during the extraction process and the subsequent vacuum concentration determine the final pigment concentrate recovery. Using microwave-assisted extraction (MAE) with ethanol resulted in a significantly higher recovery percentage of carotenoid pigment concentrate (89.53 ± 0.13%) (Figure 2a). To heat the material, MAE uses electromagnetic radiation with frequencies ranging from 0.3 to 300 GHz. This causes the molecules to rotate dipolarly and conduct ions (Camel 2001). Microwave-assisted extraction enhances bioactive compound recovery through two primary mechanisms, that is, thermal disruption and mass transfer acceleration. The absorbed microwave energy increases extraction mixture temperature, simultaneously weakening cell wall structures and reducing solvent viscosity (Kaufmann and Christen 2002; Eskilsson and Björklund 2000). This dual effect facilitates rapid diffusion of target compounds from the cellular matrix into the extraction medium, significantly improving extraction efficiency compared to conventional methods (Zhou and Liu 2006). Higher ethanol concentrations can also cause structural disruptions in cells through interactions with the phospholipid membrane of the cell, which increases the affinity for carotenoids (Jaeschke et al. 2017). Thus, the greater recovery percentage of the carotenoid pigment in the pigment concentrate was probably caused by the combination of microwave energy and ethanol as a solvent.

When ethanol was used instead of ethyl lactate, or ethanol and ethyl lactate, the carotenoid yield was noticeably higher. Better solvent accessibility to carotenoids may be the reason for the higher yield with ethanol because ethanol can degrade the complex cell wall compositions that contain carotenoids. Furthermore, the higher concentration attained with ethanol was probably caused by its greater polarity compared to that of ethyl lactate. Owing to its characteristics, ethanol can weaken and rupture cell walls, which are composed of a complex mixture of lipids, proteins and polysaccharides. More pigment can be released into the solvent as a result of this disruption, which improves carotenoid accessibility within cells. Because carotenoid pigments frequently contain polar functional groups, ethanol can dissolve and stabilise them more effectively (Silva et al. 2023). Because of its greater polarity, ethanol can enter cell structures and maintain the solubility of carotenoids in the solvent, which increases the concentration of pigment in the finished extract (Calvo et al. 2007). Ethyl lactate, on the other hand, is also a polar solvent, but it is not as polar or capable of breaking down cell walls as ethanol, which makes it less effective at removing and concentrating carotenoids from biological structures.

3.3 | Total Carotenoid, β-carotene, Lycopene and Lutein Content

The extraction method and parameters employed have a major impact on the phytochemicals extracted from plant sources. A significantly higher total carotenoid content (2069.83 ± 4.38 μg/g), as well as higher quantities of β-carotene (689.43 ± 1.04 μg/g), lycopene (1052.31 ± 0.87 μg/g) and lutein (216.96 ± 0.22 μg/g), were obtained in this study using the ethyl lactate extraction method (Figure 2b–e). The safety of end products for use in food applications is a crucial factor to consider when designing extraction techniques and technology, with a particular emphasis on choosing suitable food-grade solvents. An eco-friendly solvent that satisfies these requirements is ethyl lactate, which is produced by fermenting carbohydrate feedstocks from the maize and soybean sectors.

TABLE 1 | Compounds identified by *LC-MS/MS analysis* with retention time, mass, score, height and their health-promoting activities obtained during the representative run.

Group	Name	Retention time	Mass	Diff. (DB, ppm)	Score	Height	Health-promoting activities
Flavonoid and flavonoid derivatives	Rutaevin	6.46	486.19	3.36	70.51	95,714	Treats gastrointestinal disorders (Liu et al. 2020)
	Diginatin	8.80	796.42	0.50	98.37	113,135	—
	Myricetin 3-galactoside	7.00	480.09	3.29	91.02	108,529	MMP inhibitory, antimelanogenic, antiphotoaging and antiadipogenic effects (Oh et al. 2020)
	Corchorosol A	18.89	536.30	−6.76	69.54	315,400	Wound-healing and anti-inflammatory effects (Shady et al. 2022)
Phenolic compounds and derivatives	5-(12,15-Heneicosadienyl)-1,3-benzenediol	9.58	400.34	−4.13	88.57	99,572	Antiseptic and disinfectant (National Center for Biotechnology Information 2024)
	Syringic acid	1.85	198.05	−2.79	75.60	1292	Therapeutic applications such as antioxidant, anti-inflammatory, anticancer, antidiabetic, antiendotoxic, neuroprotective, cardioprotective and hepatoprotective effects (Srinivasulu et al. 2018)
	Caffeic acid	5.93	180.04	3.73	88.92	6446	Potential chemotherapeutic agent with antioxidant, anti-inflammatory, antiproliferative, antimicrobial and antitumour properties. Exhibits pro-oxidant-induced oxidative DNA injury, stimulating apoptotic tumour cell death (Alam et al. 2022)
	Vanillic acid	5.62	168.04	3.62	78.96	2505	Antivenom, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, free radical scavenging, antidiabetic and anticancer activities. Used in ointments, lotions and creams; fragrance, flavouring and colouring agent (Matejczyk et al. 2024).
	Ellagic acid	7.38	302.01	1.30	84.71	5308	Antioxidant, anti-inflammatory and antiproliferative; potential in treating chronic disorders, including cardiovascular diseases, neurodegenerative

(Continues)

TABLE 1 | (Continued)

Group	Name	Retention time	Mass	Diff. (DB, ppm)	Score	Height	Health-promoting activities
							diseases, diabetes and cancer (Sharifi-Rad et al. 2022)
	Rhodexin A	19.29	536.30	−5.78	79.17	130,031	Antiproliferative activity (Jung and Yoo 2011)
Terpenoids and triterpenoids	Isolimonic acid	5.74	506.21	5.95	82.19	186,362	Inhibits biofilm formation and EHEC attachment; antivirulence agent; inhibits human colon cancer cells; antiproliferative; and antiaromatase properties (J. Kim et al. 2013)
	Mangiferonic acid	9.85	454.34	0.80	96.50	101,963	Antioxidant, radioprotective, immunomodulatory, antiallergic, anti-inflammatory, antitumour, antidiabetic, lipolytic, anti-bone resorption, MAO-inhibiting, antimicrobial and antiparasitic properties (Shah et al. 2010)
	Isomasticadienonalic acid	10.08	468.32	2.29	95.30	110,406	Anti-inflammatory activity (Yueqin et al. 2003)
	Phytolaccoside A	11.06	648.39	2.45	96.02	133,418	Antioxidant, anti-inflammatory, antiparasitic, antifungal, anticancer and insecticidal effects (Bailly 2021)
	Urs-13(18)-en-3beta-yl acetate	9.34	468.40	−14.49	56.42	138,452	Anti-inflammatory, antispasmodic and antifungal properties (Oluyori et al. 2015)
Alkaloid	Tetrahydropentoxyline	5.80	366.14	1.99	98.09	156,335	Antioxidant properties (Herraiz and Galisteo 2014)
	Perlolyrine	7.74	264.09	−0.10	85.05	96,316	PDE5 inhibitor for erectile dysfunction, pulmonary hypertension and stroke; antidepressant and neuroprotective; inhibits cancer cell growth; kinase inhibitor for Alzheimer's disease and diabetes (Herraiz et al. 2023)
	23-Acetoxysoladulcidine	16.62	473.35	0.43	93.76	94,853	Antiproliferative, antibacterial, antiviral, insecticidal and antimetastatic effects (Qiu et al. 2014)
	Thalidasine	13.42	652.32	−4.88	51.23	117,996	Tumour inhibitor (Kupchan et al. 1967)
	Hovenine A	16.21	486.32	2.22	95.79	131,802	—
	Flazine	9.12	308.08	0.93	95.25	111,916	Anti-neuroinflammatory action; inhibits nitric oxide

(Continues)

TABLE 1 | (Continued)

Group	Name	Retention time	Mass	Diff. (DB, ppm)	Score	Height	Health-promoting activities
							and prostaglandin E2 production (D. Kim et al. 2016)
	Salannin	16.54	596.30	5.00	79.26	111,693	Antibacterial, anticancer, antiulcer, spermicidal and matrix metalloproteinase inhibitor properties
Glycosides and saponins	Momordicoside E	13.77	696.41	−4.48	77.96	120,061	Antibacterial and antifungal activities (Villarreal-La Torre et al. 2020)
	1-Acetyl-3,27-dihydroxywitha-5,24-dienolide 3-glucoside	10.16	662.37	0.89	98.77	110,766	—
	Calendasaponin D	7.88	1134.54	1.54	96.57	111,959	Hypolipidaemic, hypoglycaemic, antiasthmatic, antioxidant, antihypertensive and antimicrobial activity (Sharma et al. 2023)
	Cloversaponin I	13.46	632.39	2.20	95.86	116,004	Antifungal activity, hepatoprotective effects, anti-inflammatory and antioxidant properties (Ahmad and Zeb 2020)
Benzopyrans and phenylpropanoids	(5alpha,8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol	11.86	208.15	−0.19	87.11	100,630	Anti-inflammatory and antidiabetic properties (Daou et al. 2022)
	7-(4-Hydroxyphenyl)-1-phenyl-4-hepten-3-one	6.30	280.14	6.99	84.90	118,831	Ameliorates the effects of amyloid-β in Alzheimer's disease; anti-caspase and dendrite-protective; antiplatelet and nitric oxide-inhibitory effects (Elhawary et al. 2024)
	Isoferulic acid	6.00	194.06	3.33	88.54	4513	Anti-inflammatory, antiviral, antioxidant and antidiabetic properties (Jairajpuri and Jairajpuri 2016)
Macrolides	13-Deoxytedanolide	8.51	594.34	0.37	98.43	105,688	Antitumour activity; cytotoxic against P388 murine leukaemia cells (Nishimura et al. 2004)
	10-Oxo-11-octadecen-13-olide	14.88	294.22	2.15	84.18	3053	Therapeutic agent for diseases such as cancer, inflammation and autoimmune diseases (Yoshiki et al. 1996)
Fatty acid derivatives	Methyl linoleate	16.55	294.26	2.59	77.57	1638	Antifungal and antioxidant activity (Pinto et al. 2017)
	9,10-Dihydroxy-12,13-epoxyoctadecanoate	10.76	330.24	−0.21	87.72	111,656	Radical scavenging activity (S et al. 2022)

(Continues)

TABLE 1 | (Continued)

Group	Name	Retention time	Mass	Diff. (DB, ppm)	Score	Height	Health-promoting activities
Glycosylmonoacylglycerols	Gingerglycolipid B	14.15	678.38	3.49	91.81	115,379	Antioxidant, anti-inflammatory, moisturising, antiageing, antiulcer and antitumour properties (Yoshikawa et al. 1994)
	Gingerglycolipid C	15.21	680.40	1.88	96.54	114,856	Strong binding with 3CLpro of SARS-CoV-2, potential against SARS-CoV-2; strong anticancer, antitumour and antiulcer activities (Rao et al. 2022)
Diterpenoids	Cinncassiol A	7.13	382.20	2.68	87.94	130,435	Anti-inflammatory, antitumour and antibacterial properties (Zeng et al. 2014); anticomplement properties (Yagi et al. 1980)
Other compounds	Pheophorbide A	18.38	592.27	0.67	98.72	97,178	Antioxidant, anti-inflammatory, supports glucose homoeostasis via GSIS pathway; potential for diabetes management (Paul et al. 2024)
	Gravelliferone	15.35	298.16	−9.03	64.23	120,874	Antibacterial agent

The selectivity of ethyl lactate for particular carotenoids, including lutein, β-carotene and lycopene, may be the reason for the greater carotenoid content of the pigment concentrate. Because of its amphiphilic nature and intermediate polarity, ethyl lactate can interact with the restricted polar groups of carotenoids and nonpolar hydrocarbon chains. It interacts better with slightly nonpolar or hydrophobic substances because it is less polar than ethanol. Although individual carotenoids are susceptible to oxidation and heat, the milder extraction conditions for ethyl lactate, when employed without microwave-assisted extraction, help maintain their stability. The recovery of stable chemicals was significantly enhanced by the gentle extraction method. The decreased carotenoid percentage in the ethanol-extracted pigment concentrate could be because ethanol is more polar than these relatively nonpolar carotenoids, making it better suited for extracting polar chemicals. Because of this polarity mismatch, ethanol may not be as selective for specific carotenoids as ethyl lactate, even if it is more powerful at breaking through cell walls and extracting a wider variety of compounds. Similar findings were published by Kua et al. (2014), who pointed out that ethyl lactate is a versatile solvent that can dissolve substances in both hydrocarbon (nonpolar) and aqueous (polar) environments, allowing for the recovery of a variety of molecules without the need for a co-solvent. Furthermore, using ethyl lactate as a solvent, Szabo et al. (2022) observed that lycopene extraction yielded the highest values (1324.89 μg/g dry weight) in tomato processing by-products, closely followed by ethyl acetate (1313.54 μg/g dry weight). Because of its milder extraction effect and compatibility with slightly nonpolar carotenoid compounds, ethyl lactate as a solvent selectively extracts stable individual carotenoids, whereas ethanol with MAE maximises the total carotenoid yield through effective cell disruption.

3.4 | Instrumental Colour Properties

The instrumental colour values, viz., the luminosity (L*), represent how light or dark the sample is, varying from 0 (black) to 100 (white). Higher luminosity values indicate a whiter colour. Chroma a* values (*x*-axis) vary from green (−) to red (+), and chroma b* values (*y*-axis) vary from blue (−) to yellow (+). In this study, the colour values of the carotenoid pigment concentrate of gac fruit seed aril extracted with ethyl lactate indicated a rich red hue, which correlates with the presence of carotenoids, especially given that carotenoids are known to impart intense red, orange and yellow colours to various plant pigments.

The colour parameters reveal clear solvent- and method-dependent trends. Ethanol alone (T_1) yielded the highest luminosity (L* = 67.49 ± 2.05) but the lowest a* (50.84 ± 0.06), b* (36.62 ± 0.08) and chroma (57.29 ± 0.09) values. The pattern is explained by ethanol's high polarity, which effectively extracts polar carotenoids and co-extracts other light-coloured compounds, resulting in a brighter but less colour-saturated extract with a higher, more yellow-dominated hue angle (47.91 ± 0.30). Ethyl lactate alone (T_2) produced the most intense red-orange colour, with the highest a* (61.35 ± 0.20), b* (43.24 ± 0.10) and chroma (65.62 ± 0.10) values but the lowest luminosity (60.44 ± 0.12). This is because ethyl lactate's intermediate polarity and excellent solvating power preferentially extract hydrophobic, intensely coloured carotenoids such as lycopene and β-carotene, leading to high colour saturation and

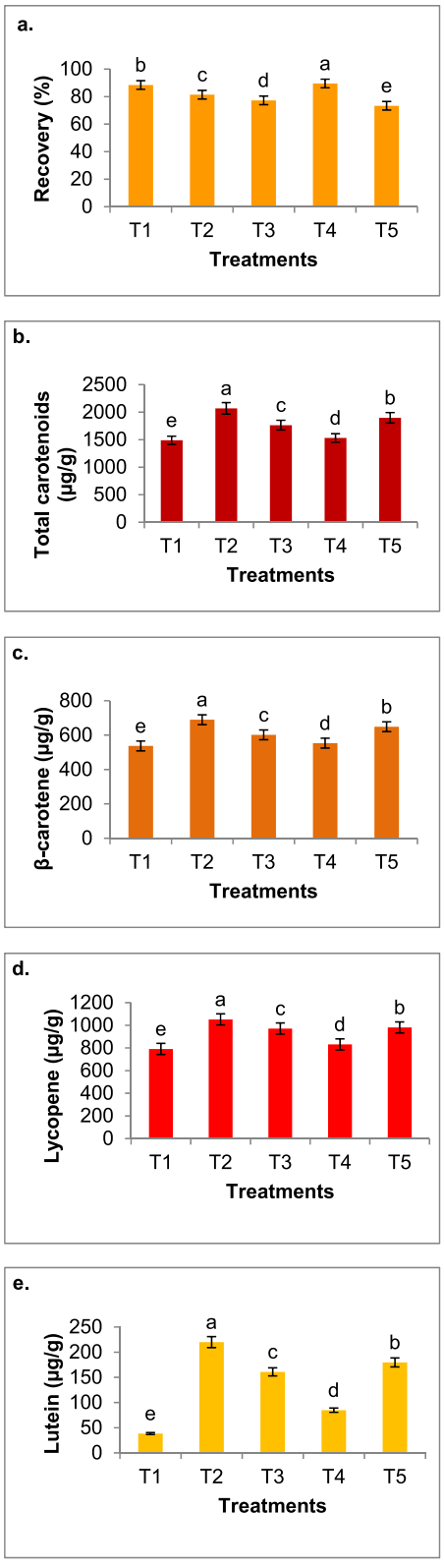


FIGURE 2 | Carotenoid recovery and content from gac seed aril using different extraction methods. (a) Carotenoid recovery from gac seed aril using different extraction methods. (b) Total carotenoid; (c) β-carotene; (d) lycopene; (e) lutein content [T_1—ethanol; T_2—ethyl lactate; T_3—ethanol and ethyl lactate (1:1 ratio); T_4—microwave-assisted extraction with ethanol; T_5—microwave-assisted extraction with ethanol and ethyl lactate (1:1 ratio)]. Similar alphabets within the column represent nonsignificant differences at ($p < 0.05$).

a lower, red-dominated hue angle (36.64 ± 0.18). The ethanol: ethyl lactate blend (T_3) balanced these effects, showing intermediate luminosity (66.38 ± 0.03) with good colour intensity (a* = 56.17 ± 0.10, chroma = 60.68 ± 0.30). The blend's widened polarity range extracts a broader carotenoid profile, combining the brightness from ethanol-soluble compounds with the colour intensity from ethyl lactate-solubilised pigments. Microwave-assisted extraction further enhanced mass transfer and cell disruption. MAE with ethanol (T_4) improved all colour metrics compared to conventional extraction (T_1), as the microwave energy facilitated the release of both polar and moderately nonpolar pigments. MAE with a combination of ethanol and ethyl lactate as the solvent (T_5) synergistically combined the microwave's efficiency with the solvent blend's broad solubility, achieving higher a* (58.60 ± 0.16) and chroma (62.41 ± 0.01) values than T_2. This demonstrates that MAE can partially offset the luminosity penalty of using a strong colour-extracting solvent (Table 2). According to Vieira et al. (2020), the combination of these colour parameters, rather than any one of them alone, correlates most significantly with carotenoid content. The success of the extraction process and the superior quality of the finished product are both reflected in the rich and stable hue of the concentrate, which is the result of this interaction (Figure 1b).

The colour parameters of gac extracts align with the commercial viability of natural colourants. Arocas et al. (2013) demonstrated that natural colourants typically exhibit lower chroma and higher lightness values compared to synthetic alternatives, characteristics that consumers perceive as more 'natural'. The gac extracts obtained in our study, particularly the ethyl lactate (T_2) with chroma 65.62 ± 0.10 and hue angle 36.64 ± 0.18, demonstrate competitive red-orange colouration within this natural colourant profile. The observed hue values suggest potential functional interchangeability with commercial red colourants, though concentration optimisation may be required to match synthetic colourant intensity. These findings indicate that gac carotenoid concentrates offer a promising balance between natural appeal and colour performance for industrial food applications.

3.5 | Total Phenolics and Antioxidant Properties (DPPH, FRAP and ABTS Assay)

The gac fruit pigment extract exhibited a remarkably high total phenolic content of 211.57 ± 0.13 mg GAE/100 g, as quantified by the Folin–Ciocalteu assay (Figure 3a,b). This significant phenolic concentration primarily stems from the presence of hydroxybenzoic acids (syringic acid and vanillic acid), hydroxycinnamic acids (caffeic acid), ellagitannins (ellagic acid) and phenolic glycosides such as rhodexin A. The antioxidant capacity of these compounds arises from their redox-active phenolic hydroxyl groups, which participate in hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms (Apak et al. 2016; Santos-Sánchez et al. 2019). High phenolic content is particularly crucial for stabilising free radicals and chelating pro-oxidant metal ions, thereby enhancing the overall antioxidant potential (Santos-Sánchez et al. 2019). Additionally, the glycosylation of certain phenolics (e.g., rhodexin A) improves their solubility, facilitating better interactions with aqueous radical species in biological systems (Trobo-Maseda et al. 2023).

TABLE 2 | Instrumental colour profile of gac fruit seed aril carotenoid pigment concentrates: extraction method comparison.

Treatments		L* value	a* value	b* value	Chroma	Hue angle
T_1	Ethanol	67.49 ± 2.05^{a}	50.84 ± 0.02^{e}	36.62 ± 0.08^{e}	57.29 ± 0.09^{e}	47.91 ± 0.30^{a}
T_2	Ethyl lactate	60.44 ± 0.12^{c}	61.35 ± 0.20^{a}	43.24 ± 0.10^{a}	65.62 ± 0.10^{a}	36.64 ± 0.18^{e}
T_3	Ethanol:ethyl lactate (1:1)	66.38 ± 0.03^{ab}	56.17 ± 0.10^{c}	40.38 ± 0.13^{c}	60.68 ± 0.30^{c}	42.58 ± 0.11^{c}
T_4	MAE with ethanol	65.96 ± 1.64^{ab}	53.70 ± 0.13^{d}	38.38 ± 0.26^{d}	58.90 ± 0.12^{d}	44.56 ± 0.10^{b}
T_5	MAE with ethanol:ethyl lactate (1:1)	63.94 ± 0.01^{bc}	58.60 ± 0.16^{b}	42.25 ± 0.10^{b}	62.41 ± 0.14^{b}	39.71 ± 0.03^{d}
S.Em±		0.18	0.13	0.15	0.17	0.17
CD (0.05)		3.57	0.41	0.45	0.51	0.52

Note: Similar alphabets within the column represent nonsignificant differences at $p < 0.05$.

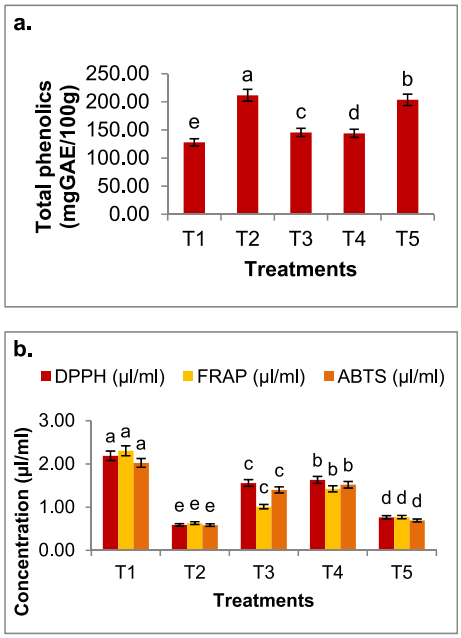


FIGURE 3 | Influence of different extraction methods on (a) total phenolics; (b) antioxidant activity of carotenoid pigment concentrate obtained from gac fruit seed aril [GAE, gallic acid equivalent; T_1—ethanol; T_2—ethyl lactate; T_3—ethanol and ethyl lactate (1:1 ratio); T_4—microwave-assisted extraction with ethanol; T_5—microwave-assisted extraction with ethanol and ethyl lactate (1:1 ratio)]. Similar alphabets within the column represent nonsignificant differences at ($p < 0.05$).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay demonstrated strong radical scavenging activity (0.59 ± 0.005 µL/mL) in gac fruit extracts, indicating efficient neutralisation of stable nitrogen-centred radicals. This activity is attributed to the hydrogen-donating ability of phenolic compounds (e.g., caffeic acid and ellagic acid) and electron delocalisation in conjugated carotenoid systems (lycopene and β-carotene) (Everett et al. 1995). Phenolics with ortho-dihydroxy groups (e.g., caffeic acid) exhibit enhanced DPPH quenching owing to their ability to form stable quinone intermediates (Charlton et al. 2023). Furthermore, the amphiphilic nature of ethyl lactate extraction ensured the optimal recovery of both hydrophilic (phenolics) and hydrophobic (carotenoids) antioxidants, maximising the radical scavenging efficiency.

The FRAP assay measures the reducing capacity of antioxidants by converting the Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ under acidic conditions (pH 3.6) (Benzie and Strain 1996). Gac fruit extracts exhibited a high FRAP value (0.63 ± 0.004 µL/mL), indicating strong electron-donating potential. This activity is predominantly driven by phenolic acids (e.g., gallic acid derivatives and caffeic acid) and flavonoids, which contain multiple hydroxyl groups capable of reducing ferric ions (Anselmi et al. 2004). The low-pH environment of the FRAP assay enhances proton-assisted electron transfer, making it particularly sensitive to polyphenols. Additionally, the presence of ellagic acid (a potent metal chelator) further amplifies ferric reduction by stabilising the oxidised intermediate. The high FRAP value confirms that gac fruit extracts are rich in secondary antioxidants, which prevent oxidative chain reactions by reducing the metal catalysts.

The ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay evaluates antioxidant capacity at neutral pH (7.4), making it suitable for assessing both hydrophilic and lipophilic antioxidants. Gac fruit extracts displayed strong ABTS scavenging activity (0.58 ± 0.005 µL/mL), primarily owing to the combined effects of phenolics, carotenoids and flavonoids (Kubola and Siriamornpun 2011). The SET mechanism dominates in this assay, where antioxidants donate electrons to stabilise the $ABTS^{\bullet+}$ radical. Carotenoids (lycopene, β-carotene and lutein) significantly contribute because of their extended π-conjugated systems, which efficiently delocalise unpaired electrons (El-Agamey et al. 2004; Pérez-Gálvez et al. 2020). Phenolic glycosides (rhodexin A) enhance solubility, ensuring effective radical quenching in aqueous environments. The strong correlation between ABTS activity and total carotenoid content underscores the importance of lipophilic antioxidants in the overall radical-neutralising capacity of gac fruit.

The antioxidant efficacy of gac fruit extracts is governed by a complex interplay between lycopene, β-carotene, lutein, phenolic compounds and other bioactive molecules identified by LC-MS/MS analysis (Le et al. 2018). Lycopene, with its 11 conjugated double bonds, exhibits superior singlet oxygen quenching and electron donation capacity, whereas β-carotene (provitamin A) contributes to radical scavenging via its 9 conjugated double bonds and potential regeneration by phenolics (Mascio et al. 1991). Lutein, a hydroxylated xanthophyll, enhances membrane protection through its polar-nonpolar structure, complementing the lipophilic antioxidant system (Charlton

et al. 2023). These carotenoids show a strong positive correlation ($R^2 > 0.90$) with ABTS activity because of their electron delocalisation capacity (Krinsky and Yeum 2003), whereas phenolic acids (e.g., ellagic acid and caffeic acid) dominate FRAP and DPPH responses via HAT/SET mechanisms. Flavonoids (myricetin 3-galactoside and rutaevin) further amplify antioxidant effects by chelating transition metals and stabilising radical intermediates, whereas terpenoids (mangiferonic acid) and alkaloids (perlolyrine) provide secondary antioxidant pathways. LC-MS/MS revealed that ethyl lactate extraction optimally preserves these interactions by maintaining the balance between hydrophilic (phenolics and glycosides) and lipophilic (carotenoids) fractions, leading to synergistic antioxidant enhancement. This multi-phytochemical network explains why gac fruit extracts outperform single-component antioxidants, offering broad-spectrum protection against oxidative stress (Le et al. 2018).

Antioxidant activity, as measured by DPPH, FRAP and ABTS assays, primarily indicates the pigment concentrate's ability to neutralise free radicals; this antioxidant capacity can help protect carotenoids from oxidative degradation, which is a major factor in colour loss.

4 | Conclusion

This study systematically compared various extraction methods to assess their impact on carotenoid yield and pigment quality from gac fruit seed aril, with the aim of identifying an optimal method for its use as a natural food colourant. Conventional solid-liquid extraction using ethanol, ethyl lactate and a combination of ethanol and ethyl lactate (1:1 ratio) and microwave-assisted extraction using ethanol, and a combination of ethanol: ethyl lactate (1:1) demonstrated distinct effects on carotenoid recovery, pigment attributes and overall antioxidant potential. Among these extraction methods, the method involving ethyl lactate (T_2) as the solvent proved to be an effective green solvent, yielding high levels of carotenoids and antioxidant potential while ensuring safety and sustainability, aligning well with industry preferences for nontoxic and eco-friendly processing methods. The extracted carotenoid pigment concentrate also indicated the presence of flavonoids, phenolic compounds, terpenoids, diterpenoids, triterpenoids, alkaloids, glycosides and saponins, benzopyrans and phenylpropanoids, macrolides, fatty acid derivatives and glycosylmonoacylglycerols having several health-beneficial properties. These findings underscore the commercial potential of gac fruit carotenoids as natural red food colourants, offering a safe, sustainable and nutritionally beneficial alternative to synthetic additives. This research provides a foundation for scalable and green extraction methods, supporting the increased use of gac fruit pigments in the food industry.

Author Contributions

N. Netravati: investigation, writing – original draft, methodology, writing – review and editing, formal analysis, data curation. **S. Gomez:** conceptualization, validation, supervision, resources, writing – review and editing. **B. Pathrose:** resources, supervision. **A. Suma:** resources. **J. Hosamani:** writing – review and editing.

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The authors have nothing to report.

Ethics Statement

The authors confirm that this study did not involve any human participants or animals, and therefore, no ethical approval or informed consent was required.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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