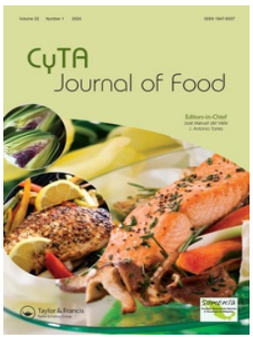

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Comprehensive biochemical profiling of coconut haustorium for innovative food industry applications

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ABSTRACT

Coconut haustorium is a tropical delicacy that mobilizes nutrients from the endosperm to nourish the germinating embryo. This study profiles coconut haustorium for sugars, organic acids, phenolics, and flavonoids using advanced chromatographic techniques. Fructose (62.70%) and glucose (18.21%) were the major sugars with negligible lactose, suggesting potential use in baby food and for lactose-intolerant individuals. Malic acid (71.79%), citric acid (12.80%), and shikimic acid (7.59%) were the prominent organic acids, contributing to the haustorium's unique taste and refreshing flavor. Ferulic acid (60.96%) and p-coumaric acid (22.05%) were the prominent phenolic acids, while naringenin (35.36%) and catechin (29.68%) were the primary flavonoids. Total phenols, flavonoids, and antioxidant activity (61.18%) were also recorded, confirming the presence of bioactive compounds. The study reveals coconut haustorium as a rich source of readily available natural sugars, organic acids, phenols, and flavonoids offering potential for developing innovative nutraceuticals and functional foods.

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KEYWORDS
Coconut haustorium; flavonoids; organic acids; phenols; sugars; antioxidant activity

Introduction

Coconut (*Cocos nucifera* L.) is a versatile and economically important crop that has significance in various aspects of life worldwide. It is a staple ingredient in the cuisines of many tropical regions, shaping local culinary traditions and adding unique flavors to dishes. Coconut provides various edible parts, including coconut water, meat, and oil. These components are rich in essential nutrients, vitamins, minerals, and dietary fiber, making them valuable for a balanced diet. West Coast Tall is a common tall variety of coconut-growing tract worldwide. It is a hardy variety with a good yield of nuts, copra, and oil and is extensively cultivated in India (Sudha et al., 2021). The germination of coconuts that have fallen due to delayed harvest and during storage for copra/oil processing was estimated to be 2% to 3% of total production, contributing to the postharvest loss (Manivannan et al., 2018). During the coconut germination process, the basal part of the embryo near the germinating pore expands to form a spongy structure called "haustorium" or "coconut apple," which fills the entire water cavity and mobilizes endosperm nutrients to nourish the germinating embryo (Smita et al., 2018).

Coconut haustorium is a nutritious tropical delicacy rich in antioxidants and various phytoconstituents with potent antibacterial and anti-inflammatory activities that can boost our immune system. It is an excellent source of essential nutrients such as proteins, vitamins, minerals, enzymes, amino acids, and bioactive compounds that can improve health benefits. It is rich in antioxidants and has immense potential to be promoted as a functional food or for food fortification (Manivannan et al., 2018). Developmental studies of coconut haustorium during germination suggested that the haustorium absorbs food materials from the solid and liquid endosperms to supply nutrients for seedling growth, thereby acting as an energy store. The nutrient content of the coconut haustorium varies with the coconut maturity, variety (Li et al., 2019), and size of the haustorium (Zhang et al., 2022). Coconut haustorium is a rich source of carbohydrates, of which the major portions are soluble sugars which need to be profiled for the efficient utilization of coconut haustorium as sugars are the essential components that contribute to nutritional, sensory, and food characteristics and serve as carriers for bioactive compounds. Organic acids in foods determine the characteristic taste and refreshing flavor profile, which enhance the consumer acceptability of fresh and processed food products.

The coconut haustorium is believed to have ample health-promoting properties and is consumed as a food material (Ramesh & Praveen, 2024). The nutritional profile of coconut haustorium is of prime importance for its efficient utilization in dietary formulations and as a supplement. The previously published studies cover a range of biochemical components from locally collected coconut haustorium and focus mainly on phenols and flavonoid profiling, with limited work on sugar and organic acid profiling. To the best of our knowledge, there are only a few reports on sugar and acid profiling of coconut haustorium (Li et al., 2019). A comprehensive knowledge of the biochemical constituents in coconut haustorium is essential for optimizing its

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utilization in functional foods and the food industry. However, various phytochemicals in coconut haustorium have been little studied using reliable analytical technology. Thus, a detailed study on the composition and content of various phytochemicals in coconut haustorium is needed to utilize it as an ingredient for functional foods (Zhang et al., 2022). Liquid chromatography-mass spectrometry has been widely used for screening chemicals in plants since it provides excellent accuracy in identifying chemical structures. Hence, the study was conducted with the objective of investigating the compositions and contents of sugars, organic acids, phenolic acids, and flavonoids in coconut haustorium through liquid chromatography-tandem mass spectrometry (LC-MS/MS), and also to characterize bioactive compounds (total phenols, total flavonoids) and to assess the antioxidant activity which is necessary to understand its nutritional components for novel food applications.

Materials and methods

Collection of coconut haustorium

Coconut haustorium at optimal maturity (2 months after germination, outer layer yellowish creamy and inner white portion spongy without fibrous development) from germinated seeds of the coconut variety West Coast Tall (WCT) was harvested after dehusking, deshelling, and ozonizing at two ppm for sanitization. The haustorium was cut uniformly to incorporate yellow and white spongy portions, dried in a cross-flow cabinet tray drier at 50°C until the moisture reached 8%, and then ground to a fine powder. Coconut haustorium powder was further used to profile sugars, organic acids, phenolic acids, and flavonoids in triplicate.

Profiling of sugars

The sugars were extracted using the method described by Steppuhn and Wackers (2004), where 2 g of sample were extracted with 12 mL warm (40°C to 50°C) 80% ethanol twice, collecting the supernatant to a final volume of 25 mL. From this, 10 mL of extract was evaporated to dryness in a water bath at 80°C, and the residue was dissolved in 2 mL of 0.01% formic acid. The mixture was sonicated for 10 min, followed by extraction with ethyl acetate 2 to 3 times, which was completely removed by keeping it in a water bath at 60°C to 70°C for 30 min. Subsequently, the volume was assembled using the mobile phase, which combined solvents A (80:20-Acetonitrile: Water) and B (30:70-Acetonitrile: Water + 0.1% ammonium hydroxide) in 1:1 ratio. It was then filtered and introduced into the Waters UPLC (Ultra Performance Liquid Chromatography) H class system equipped with the TQD MS/MS system for analysis. A Triple Quadrupole Mass Spectrometer (TQD MS/MS) is a tandem mass spectrometer/detector that combines liquid chromatography (LC) and tandem mass spectrometry (MS/MS), offering accurate quantification of targeted compounds.

LC-MS/MS conditions

The initial gradient was 100% solvent A (80:20-Acetonitrile: Water), held for 1 min. At 8 min, the gradient was changed to 88% of solvent A and 12% of solvent B (30:70-Acetonitrile: Water + 0.1% ammonium hydroxide), held for 1 min, and a linear gradient followed by 98% solvent A and 2% solvent B at 15 min, held for 0.5 min. The system was then returned to the initial conditions at 19 min and equilibrated for 1 min before the next injection. The flow rate was 0.1 mL/minute, and the analytical column was a 2.1 × 100 mm UPLC BEH-Amide column (Waters, U.S.A.) with 1.7 µm particles protected by a vanguard BEH-Amide with 1.7 µm. The guard column (Waters, U.S.A.) was used with a column temperature of 25°C. The elution was monitored using a PDA detector, and the UPLC column effluent was pumped directly without any separation into the TQD-MS/MS (Waters, U.S.A.) system, optimized for sugar analysis.

Profiling of organic acids

Extraction procedure

The extraction procedure of the organic acids followed was described by Oliveira et al. (2008). The coconut haustorium sample (5 g) was homogenized using 10 mL of 80% methanol and sonicated for 30 min. The extract was then allowed to cool at room temperature and centrifuged at 11,178 g for 15 min. The supernatant was collected, and the traces of methanol were completely removed by a vacuum evaporator. The extract was then transferred to a separatory funnel and extracted 3–4 times with 25 mL of ethyl acetate. The lower aqueous phase was collected, traces of organic solvent were completely removed using a vacuum evaporator, and the concentrated sample was reconstituted with water for SPE purification (Solid Phase Extraction, which is regularly used for separating and purifying food-derived oligosaccharides and peptides prior to liquid chromatography-mass spectrometry (LC-MS/MS) analysis where water is used as purification solvent). The eluted solution from the cartridge was dried completely under the nitrogen flow. After being reconstituted in the mobile phase, solvent A and solvent B in 50:50 (solvent A:10 mm Ammonium acetate: Acetonitrile (50:50) and solvent B (Acetonitrile with 0.05% formic acid)), the mixture was filtered through a 0.45 µm-pore nylon membrane filter, followed by filtration through a 0.2 µm nylon membrane filter and further injection of 4 µL of the final solution was done into the LC-MS/MS for the measurement of organic acids.

LC-MS/MS conditions

The initial gradient composed of 100% aqueous phase (A [10 mm Ammonium Acetate: Acetonitrile (50:50)]) and organic phase 0% (B [Acetonitrile with 0.05% formic acid]) were held for 0.5 min. At 5.0 min, the gradient was changed to a 95% aqueous phase and 5% organic phase, held for 0.5 min, and then the system was returned to the initial conditions at 6 min. This condition was held for 1 min to equilibrate before the next injection. The flow rate was 0.1 mL/minute. The analytical column used was a 2.1 × 50 mm UPLC BEH – Amide column (Waters) with 0.7 µm particles, protected by a Vanguard 2.1 × 5 mm BEH-Amide column with 0.7 µm particle size guard column (Waters, U.S.A.) and the column temperature was maintained at 25°C. The injection volume of the sample was 4 µL. The eluted organic acids were monitored using Photo Diode Array (PDA) detector which collects the entire spectrums and allows the absorbance at multiple wavelengths to be monitored simultaneously; and the UPLC column effluent was pumped directly without any splitting into the TQD-MS/MS (Waters,

U.S.A.), which was optimized for identifying and quantifying organic acid analysis.

Profiling of phenols and flavonoids

Individual phenolic acids and flavonoids for LC-MS/MS analysis were isolated following the methodologies for metabolite extraction and analysis as described by Chen et al. (2001) and Kanade et al. (2022). The coconut haustorium sample (10 g) was homogenized in 80% methanol, centrifuged, and made up to 50 mL. An extract of 20 mL was evaporated close to dryness under a vacuum at 45°C. It was diluted to 5 mL with water, and extracted three times with petroleum ether, followed by 40 mL of ethyl acetate using a separatory funnel. The aqueous layer was discarded, and ethyl acetate extract was evaporated to dryness under vacuum at room temperature. The dry residue was mixed with 4 mL of 2N NaOH and allowed to hydrolyze overnight. After acidification to pH 2 using 5 mL of 1N HCl, it is re-extracted with 50 mL of ethyl acetate. The ethyl acetate layer was re-extracted twice with 25 mL of 0.1N $NaHCO_3$. The ethyl acetate layer carrying the flavonoids was evaporated to complete dryness under a vacuum, and the residue dissolved in 2 mL of MS-grade methanol was filtered through a nylon filter before injection in LC-MS/MS for the estimation of flavonoids.

The aqueous layer was further acidified to pH 2 with 5 mL of 2N HCl and extracted three times with 25 mL of ethyl acetate in order to prepare the extract for phenolic acid profiling. The ethyl acetate layer was completely dried in a rotary evaporator, and the residue was dissolved in 2 mL of MS-grade methanol and filtered through a 0.2 µm nylon filter before injection in LC-MS/MS for the estimation of phenolic acids.

LC-MS/MS conditions

Phenolic acids and flavonoids were resolved in the analytical column BEH-C18 (2.1 × 50 mm, 1.7 µm) from Waters India Ltd., protected by a Vanguard BEH C-18 (Waters, U.S.A.) with gradient flow of the organic and aqueous phase with a flow rate of 0.3 mL/min. The column temperature was maintained at 25°C during the analysis, and the sample injection volume was 2 µL. The eluted phenolic acids and flavonoids were monitored by a PDA detector, and the UPLC column effluent was pumped directly without any splitting into the TQD-MS /MS (Waters, U.S.A.) system optimized for the phenolic acids and flavonoids analysis.

Estimation of total phenols

Total phenol content was estimated by adopting the method described by Aparna and Lekshmi (2023). The dried coconut haustorium (1 g) was extracted with ten times the volume of 80% ethanol. The homogenate was centrifuged at 11,178 g for 20 min. The supernatant was evaporated to dryness, and the residue was added with a known volume of distilled water (5 mL). An aliquot (0.2 mL), taken in a test tube, was made up to 3 mL with distilled water, followed by adding 0.5 mL Folin – Ciocalteau reagent and 2 mL Na_2CO_3 (20%) after 3 min. The test tubes were cooled in boiling water for 1 min, and the absorbance was measured at 650 nm against the reagent blank. A standard curve was plotted at different concentrations of Gallic acid, and the phenol content of the sample was expressed as mg total phenols in Gallic Acid Equivalent (mg GAE 100 g^{-1}) and measured in three replicates.

Estimation of total flavonoids

The total flavonoid content of dried haustorium samples was estimated according to the colorimetric assay described by Quettier-Deleu et al. (2000) and Aparna and Lekshmi (2023). The ethanolic extract (1 mL) of coconut haustorium (as in the estimation of total phenols) was mixed with 5 mL distilled water, and 0.3 mL $NaNO_2$ (5%) was added to the solution and kept for 5 min, followed by the addition of 0.3 mL $AlCl_3$ (10%) which was held for 6 min. The resultant solution was mixed with 2 mL 1 M NaOH, and the final volume was made up to 10 mL using distilled water. The solution was mixed vigorously, and the absorbance was measured at 510 nm. The flavonoid content was expressed in µg Quercetin equivalent per gram (µg QE g^{-1}) of the sample (in three replicates) compared with the quercetin standard curve, plotted under the same conditions without the sample.

Estimation of antioxidant activity

The total antioxidant activity of coconut haustorium was determined using a 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as described by Sharma and Bhat (2009). The coconut haustorium (in three replicates) was extracted with 80% ethanol (1:10 w/v) and the extract was allowed to react with DPPH solution (1:2 v/v) in darkness for 30 min. The scavenging effect on DPPH free radicals was calculated by measuring the absorbance at 517 nm and was expressed as % inhibition of DPPH determined by subtracting the absorbance of the DPPH with sample from the absorbance of the DPPH solution without sample, dividing the result by the absorbance value of DPPH solution without sample, and multiplying by 100. This percentage inhibition reflects the sample's ability to neutralize DPPH free radicals.

Statistical analysis

The results were reported as Mean ± SD (standard deviation) in three replicates. The analysis of variance (ANOVA) was conducted, and treatment means were compared ($p < .05$) using Fishers Protected Least Significant Difference test. The concentrations of identified different sugars, organic acids, phenolic acids, and flavonoids were also expressed as a percentage of their total concentration. To convert these values to percentages, we considered the given concentrations as they were, without further conversion. The percentage for each individual component was calculated using the following formula: Percentage (%) = (Concentration of individual component/Total concentration) × 100. The total concentration was calculated as the sum of individual component concentrations for each profiling. The results were rounded to two decimal places for consistency.

Results and discussion

A schematic overview of the study and its findings is depicted in Figure 1.

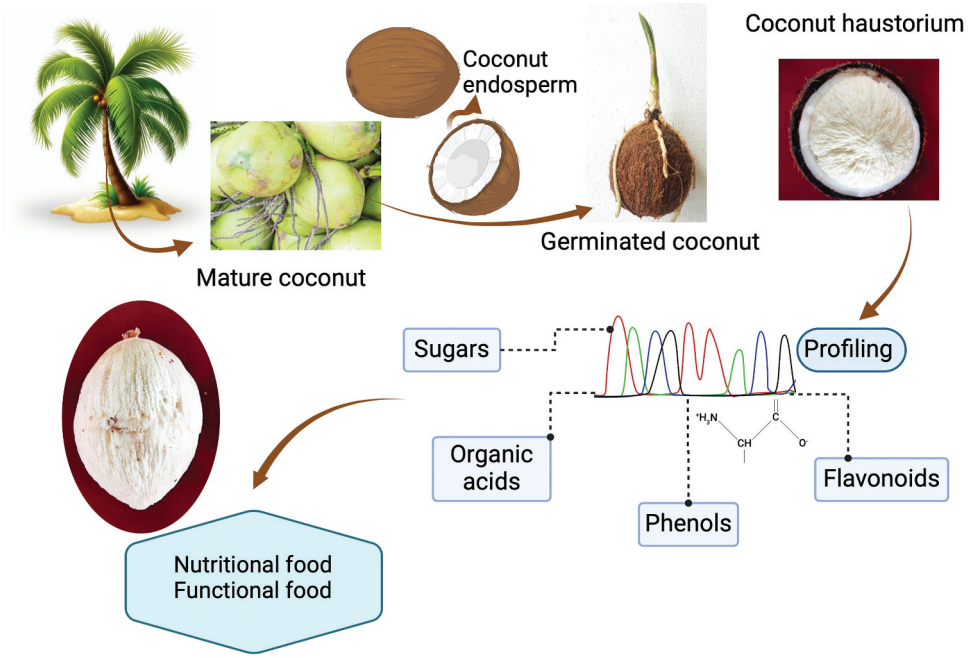

Figure 1. Overview of study design and key findings in phytochemical profiling of coconut haustorium.

Sugars

Sugars serve as the main source of readily available energy, and the sugars present in coconut haustorium make it a convenient, ready-to-eat snack and also for the development of other processed products. The major sugar present in coconut haustorium (Table 1 and Figure 2) was found to be fructose (314.83 ± 2.25 mg g^{-1}), followed by glucose (91.45 ± 0.08 mg g^{-1}) and mannose (37.30 ± 0.34 mg g^{-1}). The fructose contributed 62.70% of the total sugars identified and quantified in the profiling study, whereas 18.21% was glucose 7.43% as mannose. The sucrose content in coconut haustorium was 24.36 ± 0.22 mg g^{-1} and sorbitol (22.77 ± 0.20 mg g^{-1}), inositol (10.63 ± 0.26 mg g^{-1}) and ribose; galactose; fucose were present in smaller amounts. These findings were consistent with the observations recorded by Li et al. (2019), where the main sugars present in coconut haustorium were reported as fructose and glucose in Hainan Tall and Red Dwarf varieties of coconut in China, and the sugar content in haustorium varied with the variety, sugars of solid and liquid endosperm, and seedling growth stages. The dried coconut haustorium was reported as a good source of total and reducing sugars (Manivannan et al., 2018). The three main sugar components, *viz*. fructose, glucose, and sucrose, were detected in coconut sugar syrup (Asghar et al., 2020) whereas the sugar profiling study carried out for coconut water by Bandupriya et al. (2022) recorded sucrose, glucose, galactose, and fructose as the major sugars. Coconut haustorium has higher reducing sugars and can be used as a food supplement for lactose-intolerant children and preparation of confectioneries (Manivannan et al., 2018). In this sugar profiling study of coconut haustorium, major sugars were identified as fructose and glucose which are the readily available primary sources of energy that give a refreshing feel to the coconut haustorium. This natural sweetness contributes to the pleasant taste and flavor profile and enhances the palatability and acceptance of food products. The current study also revealed that

Table 1. Sugar and organic acids in coconut haustorium.

Sugars (mg g^{-1})		Percentage	Organic acids (mg g^{-1})		Percentage
Ribose	0.24 ± 0.00^{g}	0.05	Lactic acid	0.03 ± 0.00^{h}	Negligible
Arabinose	0.05 ± 0.00^{g}	Negligible	Pyruvic acid	3.41 ± 0.22^{e}	1.71
Xylose	0.05 ± 0.00^{g}	Negligible	Malonic acid	4.35 ± 0.08^{d}	2.18
Rhamnose	0.03 ± 0.00^{g}	Negligible	Maleic acid	0.19 ± 0.00^{h}	0.10
Fucose	0.11 ± 0.00^{g}	0.02	Fumaric acid	1.17 ± 0.01^{g}	0.59
Fructose	314.83 ± 2.25^{a}	62.70	Succinic acid	4.09 ± 0.23^{d}	2.05
Glucose	91.45 ± 0.08^{b}	18.21	Malic acid	143.34 ± 0.57^{a}	71.79
Mannose	37.30 ± 0.34^{c}	7.43	Tartaric acid	0.02 ± 0.00^{h}	Negligible
Galactose	0.22 ± 0.008	0.04	Shikimic acid	15.16 ± 0.07^{c}	7.59
Inositol	10.63 ± 0.26^{f}	2.12	Citric acid	25.56 ± 0.48^{b}	12.80
Sorbitol	22.77 ± 0.20^{e}	4.53	Hydroxycitric acid	2.35 ± 0.18^{f}	1.18
Sucrose	24.36 ± 0.22^{d}	4.85			
Maltose	0.08 ± 0.01^{g}	0.02			
Trehalose	0.01 ± 0.00^{g}	Negligible			
Lactose	0.03 ± 0.00^{g}	Negligible			

Values are given as mean ± standard deviation of three replicates.

Different letters in the column for each parameter indicate significant differences among the treatments ($p < .05$) as per Least Significant Difference test.

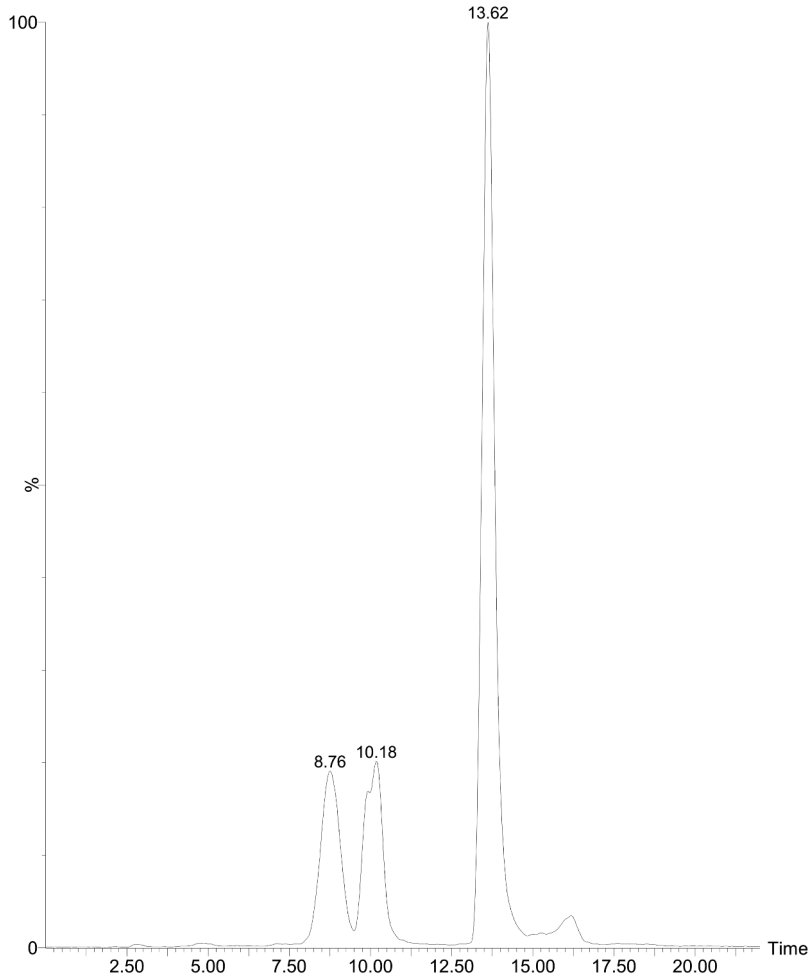

Figure 2. Chromatogram of sugars in coconut haustorium profiled by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The peak represents different sugars and retention time along the X-axis.

coconut haustorium contains a negligible amount of lactose; hence, it can be effectively used in baby food formulations or products for lactose-intolerant patients and nutraceuticals.

Organic acids

Organic acids are naturally occurring compounds in various fruits and vegetables, contributing to their taste, aroma, and nutritional profile. They play several important roles in human health. The organic acids profiled from coconut haustorium are presented in Table 1, and the chromatogram is presented in Figure 3. The major organic acid present in coconut haustorium was observed as malic acid 143.34 ± 0.57 mg g^{-1}, which contributed to 71.79%, followed by citric acid (25.56 ± 0.48 mg g-1) at 12.80% and shikimic acid (15.16 mg g^{-1} ±0.07) recorded as 7.59% of the total organic acids identified. Among the acids quantified, malonic acid (4.35 ± 0.08 mg g^{-1}), succinic acid (4.09 ± 0.23 mg g^{-1}), pyruvic acid (3.41 ± 0.22 mg g^{-1}), hydroxycitric acid (2.35 ± 0.18 mg g^{-1}) and fumaric acid (1.17 ± 0.01 mg g^{-1}) were also present in smaller quantity. Studies on the organic acid profiling of coconut haustorium are significantly less, and this is the first in this way as best of our search. The malic and citric acids were reported as naturally present in mature coconut pulp (Mauro & Garcia, 2019) and aconitic acid, malic acid and succinic acid were reported in tender coconut water (Gomez-Tah et al., 2023). The present study revealed malic acid as the major organic acid, followed by citric acid, which provides a characteristic taste and flavor profile that enhances sensory attributes of fresh and processed products of coconut haustorium revealing its potential for the use in food industry.

Phenolic acids

Phenolic acids are groups of phytochemical compounds found in various plant-based foods and have garnered considerable attention due to their potential health benefits. Phenolic acids may individually or synergistically contribute to antioxidant activities and explain antioxidant, antimutagenic, antitumor, and anticarcinogenic properties (Neo et al., 2010). Gallic and caffeic acids are phenolic compounds with relatively high antioxidant properties in many fruits and vegetables (Khuwijitjaru et al., 2014). The present profiling study of coconut haustorium revealed the presence of 18 phenolic acids, of which ferulic acid was the major (2035.58 ± 0.91 µg g^{-1}), followed by p-coumaric acid (736.23 ± 0.58 µg g^{-1}) and o-coumaric acid (356.70 ± 3.19 µg g^{-1}) (Table 2; Figure 4). The ferulic acid contributed to 60.96% of the total phenolic acids quantified, and p-coumaric acid was 22.05%. The other abundant phenolic acids present in coconut haustorium were gentisic acid (87.87 ± 0.42 µg g^{-1}), protocatechuic acid (56.57 ± 0.32 µg g^{-1}), t-cinnamic acid (17.15 ± 0.62 µg g^{-1}), caffeic acid (12.50 ± 0.77 µg g^{-1}) and salicylic acid (10.23

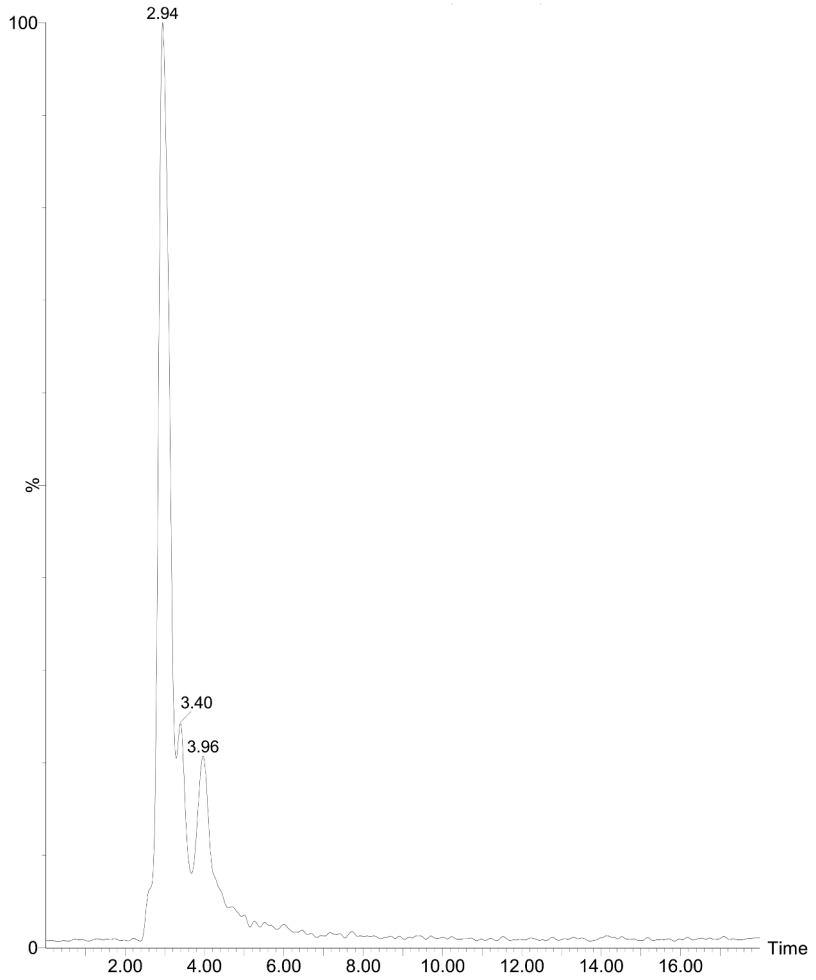

Figure 3. Chromatogram of organic acids in coconut haustorium profiled by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The peak represents different organic acids and retention time along the X-axis.

Table 2. Phenolic acids and flavonoids in coconut haustorium.

Phenolic acids (μg g^{-1})		Percentage	Flavonoids (μg g^{-1})		Percentage
Benzoic acid	2.25 ± 0.13^{jk}	0.07	Umbelliferone	0.01 ± 0.00^{h}	0.02
p-hydroxy benzoic acid	3.57 ± 0.11^{ijk}	0.11	Apigenin	3.31 ± 0.08^{c}	12.67
Salicylic acid	10.23 ± 0.42^{h}	0.31	Galangin	0.02 ± 0.00^{h}	0.09
3-Hydroxy benzoic acid	3.89 ± 0.05^{i}	0.12	Naringenin	9.24 ± 0.06^{a}	35.36
t-Cinnamic acid	17.15 ± 0.62^{f}	0.51	Kaemperol	0.01 ± 0.00^{h}	0.05
2,4-dihydroxybenzoic acid	2.18 ± 0.53^{k}	0.07	Luteolin	1.62 ± 0.05^{d}	6.21
Gentisic acid	87.87 ± 0.42^{d}	2.63	Fisetin	0.004 ± 0.00^{h}	0.02
Protocatechuic acid	56.57 ± 0.32^{e}	1.69	Eriodictyol	0.001 ± 0.00^{h}	Negligible
p-Coumaric acid	736.23 ± 0.58^{b}	22.05	Catechin	7.75 ± 0.03^{b}	29.68
o-Coumaric acid	356.70 ± 3.19^{c}	10.68	Epicatechin	1.18 ± 0.08^{e}	4.52
Vanillic acid	3.66 ± 0.23^{ij}	0.11	Hesperetin	0.06 ± 0.00^{h}	0.24
Gallic acid	9.34 ± 0.46^{h}	0.28	Quercetin	1.22 ± 0.07^{e}	4.66
Caffeic acid	12.50 ± 0.77^{g}	0.37	Epigallocatechin	0.62 ± 0.02^{f}	2.38
Ferulic acid	2035.58 ± 0.91^{a}	60.96	Myricetin	0.55 ± 0.04^{fg}	2.11
Syringic acid	0.56 ± 0.02^{l}	0.02	Rutin	0.52 ± 0.02^{g}	1.99
Sinapic acid	0.61 ± 0.04^{l}	0.02			
Ellagic acid	0.04 ± 0.00^{l}	Negligible			
Chlorogenic acid	0.003 ± 0.00^{l}	Negligible			

Values are given as mean ± standard deviation of three replicates.

Different letters in the column for each parameter indicate significant differences among the treatments ($p < .05$) as per Least Significant Difference test.

± 0.42 μg g^{-1}). Phenolic acids such as gallic acid (9.34 ± 0.46 μg g^{-1}), 3-hydroxy benzoic acid (3.89 ± 0.05 μg g^{-1}), vanillic acid (3.66 ± 0.23 μg g^{-1}), p-hydroxy benzoic acid (3.57 ± 0.11 μg g^{-1}), benzoic acid (2.25 ± 0.13 μg g^{-1}), 2,4 dihydroxybenzoic acid (2.18 ± 0.53 μg g^{-1}), sinapic acid (0.61 ± 0.04 μg g^{-1}), syringic acid (0.56 ± 0.02 μg g^{-1}), ellagic acid (0.04 ± 0.00 μg g^{-1}), and chlorogenic acid (0.003 ± 0.00 μg g^{-1}) were also present in coconut haustorium. Xia et al. (2011) reported that phenolic acids such as caffeic acid, p-coumaric acid, protocatechuic acid, and gallic acid were present in coconut sap. Thirteen phenolic acids were identified from coconut sap, of which ferulic acid, vanillic acid, p-hydroxybenzoic acid, and trans-cinnamic acid were the main phenolic acids (Hebbar et al., 2020). The size of the coconut haustorium influenced its nutrient composition and phenolic content. The highest concentration was recorded in small haustorium rather than mid and large-sized ones in Indonesian coconuts (Zhang et al., 2022). The profiling study

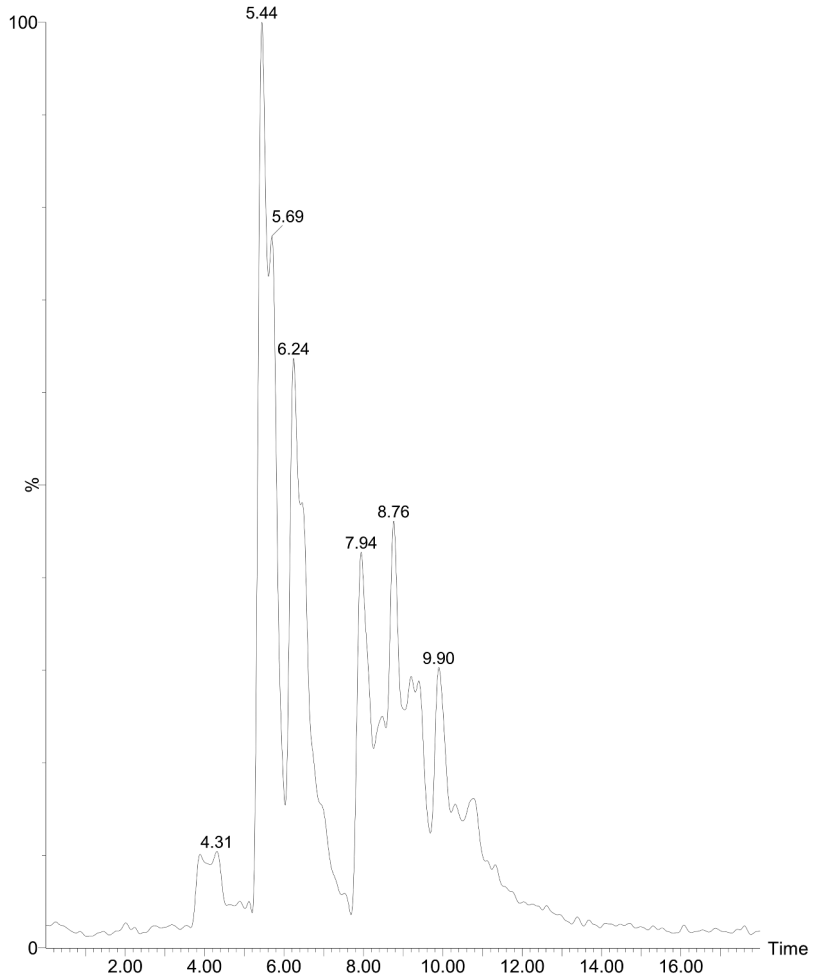

Figure 4. Chromatogram of phenolic acids in coconut haustorium profiled by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The peak represents different phenolic acids and retention time along the X-axis.

of haustorium by Kim et al. (2023) reported 4.27 mg 100 g^{-1} of ferulic acid and Narayanankutty et al. (2023) recorded ferulic acid as the major phenolic acid, followed by p-coumaric acid, gallic acid, o-coumaric acid, and trans-cinnamic acid. The present profiling study of coconut haustorium of the West Coast Tall also reported ferulic acid, p-coumaric, and o-coumaric acid as the major phenolic acids, but the gallic acid concentration was lesser, and several other phenolic compounds were also identified and quantified, which might be due to the varietal difference or growing conditions. Hence, the current study revealed the potential of coconut haustorium in the functional food industry for developing products with cardioprotective, anti-diabetic, and anti-inflammatory properties.

Flavonoids

Flavonoids exhibit good free radical scavenging activity and are responsible for antioxidant and anti-inflammatory properties. These secondary metabolites are part of polyphenols (Indiarto et al., 2024). As of our knowledge, the identification of flavonoids in coconut haustorium has not been reported, barring one exception (Kim et al., 2023). The flavonoid compounds in coconut haustorium were profiled (Table 2 and Figure 5). Among the 15 identified flavonoid compounds, naringenin was the key flavonoid (9.24 ± 0.06 µg g^{-1}) with 35.36% followed by catechin (7.75 ± 0.03 µg g^{-1}) at 29.68%, and apigenin (3.31 ± 0.08 µg g^{-1}), luteolin (1.62 ± 0.05 µg g^{-1}), quercetin (1.22 ± 0.07 µg g^{-1}) and epicatechin (1.18 ± 0.08 µg g^{-1}) were also observed. The flavonoids *viz*., myricetin (36.30 mg 100 g^{-1}), catechin, kaempferol, and hesperetin were reported in coconut haustorium from the Vietnamese coconuts and also recorded two large unknown peaks while profiling and recommended for further studies (Kim et al., 2023). In the present study of coconut haustorium from the West Coast Tall variety, naringenin was the main flavonoid, followed by catechin, apigenin, luteolin, and quercetin, pointing out the varietal variation in biochemical composition and the unidentified peaks recorded in Vietnamese coconut haustorium by Kim et al. (2023) can be checked for the flavonoids recorded in the present study. Catechin, quercetin, and epigallocatechin were recorded as the flavonoids in coconut oil, contributing to its antioxidant activity (Srivastava et al., 2016). The studies conducted by Arivalagan et al. (2018) in coconut testa reported flavonoid compounds that belong to flavan-3-ol (catechin, epicatechin and epigallocatechin), flavonol (myricetin, quercetin and kaempferol), glycoside (rutin), flavone (luteolin and apigenin), flavanone (naringenin and hesperetin) and coumarin derivative (umbelliferone) supporting the results of this investigation. Even though the constituents of total phenol and flavonoids vary with variety and maturity, coconut haustorium is a potent source of bioactive compounds that contribute to the antioxidant properties and reduce the risk of chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative diseases.

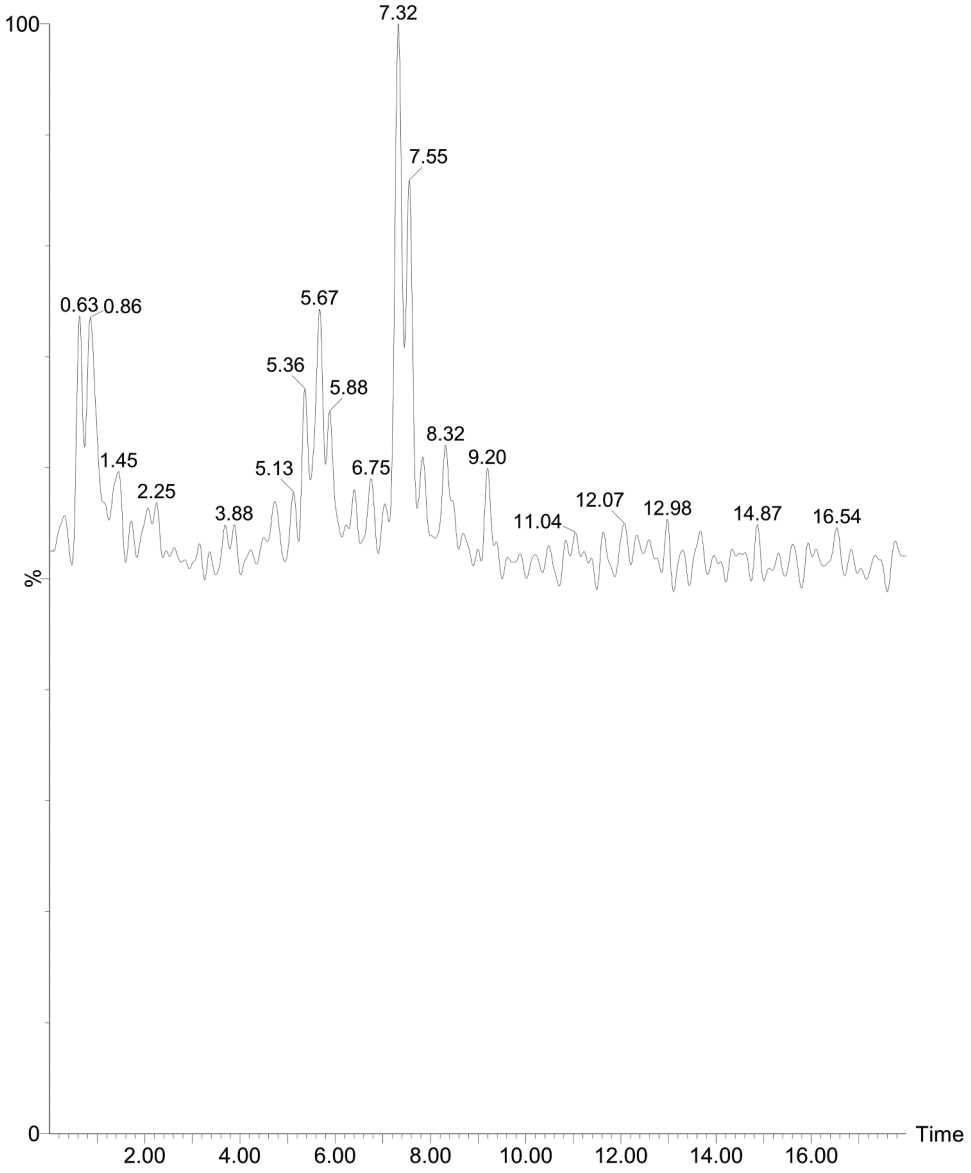

Figure 5. Chromatogram of flavonoids in coconut haustorium profiled by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The peak represents different flavonoids and retention time along the X-axis.

Total phenols, total flavonoids and antioxidant activities

The colorimetric quantification of total phenols, flavonoids, and antioxidant activity of coconut haustorium confirmed its nutritional importance. The coconut haustorium recorded a total phenolic content of 185.87 $\pm$ 4.27 mg GAE 100 g^{-1} on a dry weight basis, which was in accordance with the reports of total phenolic content of unpeeled and peeled coconut haustorium flour (Smita et al., 2018). In contrast, lower values of total phenol in coconut haustorium in the West Coast Tall coconut variety (146 mg GAE 100 g^{-1}) were reported by Manivannan et al. (2018) and Kim et al. (2023) in Vietnamese coconut (83.20 mg GAE 100 g^{-1}). The total phenol content in the present study was higher than in the above studies, which might be due to the varietal difference and variation in the maturity of coconut haustorium. The total phenol content in coconut water (5.18 to 7.71 mg GAE 100 mL^{-1}) and coconut meat (6.28 to 10.00 mg GAE 100 g^{-1}), from which the coconut haustorium develops during germination, was lower than the haustorium (Mahayothee et al., 2016). This highlights the physiological and biochemical changes that occur during haustorium development that warrant detailed study. The quantification of total flavonoid recorded 179.25 $\pm$ 5.66 µg QUE g^{-1}. In contrast, a lower value was observed as 2.36 mg QE 100 g^{-1} in methanolic extract and 1.16 mg QE 100 g^{-1} in chloroform extract (Kannaian et al., 2020) and 73.50 mg QE 100 g^{-1} in 70% methanolic extract of coconut haustorium by Kim et al. (2023).

The phytochemicals present in coconut haustorium help in scavenging the free radicals produced in the human body due to metabolic processes and life stress. The antioxidant activity as a percentage of inhibition of free radicals was recorded at 61.18 $\pm$ 3.18%, which showed the potential of coconut haustorium as a good supplement for natural antioxidants. This might be due to the presence of bioactive compounds mainly phenols and flavonoids. The plant's secondary metabolites, phenols, and flavonoids are effective free radical scavengers and potent antioxidants. These are positively associated with the antioxidant properties of food. The colorimetric analysis and profiling of phenolic acids and flavonoids of coconut haustorium conducted in this study

revealed its richness in the bioactive compounds. The synergistic effect of phenols and flavonoids contributed to the potential antioxidant activity of coconut haustorium, which can be utilized for the development of functional or nutraceutical foods. Similar reports of the antioxidant activity of coconut haustorium were reported by Manivannan et al. (2018) as 72.80%, and a lower value of 27.67% was stated by Narayanankutty et al. (2023). The antioxidant potential varies with the variety, size, and maturity of coconut haustorium Zhang et al. (2022), and further extraction and identification of compounds responsible for antioxidant properties need to be explored.

Conclusions

Today, consumer preference is shifting toward healthy and natural food, and the food industry focuses on novel and nutritionally improved food for human well-being. Our study on the comprehensive profiling of coconut haustorium identified fructose (314.83 mg g^{-1}), glucose (91.452 mg g^{-1}), and mannose (37.30 mg g^{-1}) as the major sugars with a percentage contribution of 62.70%, 18.21%, 7.43% respectively with negligible amounts of lactose. The prominent organic acid was recorded as malic acid 143.34 mg g^{-1}, followed by citric acid (25.56 mg g^{-1}) and shikimic acid (15.16 mg g^{-1}) in coconut haustorium. Malic acid was identified as the major organic acid in coconut haustorium, with 71.79% of the total acid concentration. The composition of these simple sugars and organic acids contributes to the peculiar taste and flavor of the coconut haustorium, making it a tropical delicacy that opens opportunities for processed products. The phenolic acid profiling of coconut haustorium identified and quantified 18 phenolic acids, with ferulic acid (2035.58 µg g^{-1}) being the most abundant (60.96%), followed by p-coumaric acid (736.23 µg g^{-1}) and o-coumaric acid (356.70 µg g^{-1}). The study also identified and quantified 15 flavonoid compounds, of which naringenin was the main flavonoid (9.24 µg g^{-1}) contributing to 35.36% of total flavonoid compounds identified, followed by catechin (7.75 µg g^{-1}) and apigenin (3.31 µg g^{-1}). Coconut haustorium contains very little lactose and has potential in the food industry for developing baby foods and nutraceuticals for lactose-intolerant patients. Consumption of coconut haustorium can prevent degenerative diseases related to oxidative stress and can be explored as a food supplement, functional foods, or food fortification. Further research is needed, as the composition and bioactive compounds in coconut haustorium depend on the variety and maturity to determine the ideal stage of consumption and utilization as a functional food. The unique blend of available sugars and organic acids of coconut haustorium and the richness of bioactive compounds, phenolic acids, and flavonoids with promising antioxidant potential make it an invaluable natural source for developing innovative products with enhanced nutritional quality, flavor, and shelf stability.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data supporting this study's findings are available from PRGL, one of the corresponding authors, upon reasonable request.

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