IJBPAS, October, 2026, 15(10): 4910-4919

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL CHARACTERISATION AND HPLC QUANTIFICATION OF KEY BIO ACTIVE PHENOLICS IN *CARISSA CARANDAS*

G. NAGARAJU[1*], V. PHANINDRA[1], SK. ABUBAKAR SIDDIQ[1], R. BALARAJU[1], MD. ADIL HUSSAIN[1], MD. ALTHAF HUSSAIN[1] AND N. A. SAI GANESH[1]

[1]Chalapathi Institute of Pharmaceutical Sciences, Guntur, A.P 522034, India

***Corresponding Author: Dr. Gollapalli Nagaraju: E Mail: rajaneeraja@gmail.com**

Received 25th Dec. 2025; Revised 26th Jan. 2026; Accepted 15th May 2026; Available online 1st Oct. 2026

https://doi.org/10.31032/IJBPAS/2026/15.10.10532

ABSTRACT

Karonda, or *Carissa carandas,* is a fruit that is widely found in tropical and subtropical areas and has significant nutritional and medicinal value **[1, 2]**. Because of their antimicrobial, anti-inflammatory, and antioxidant qualities, gallic acid and chlorogenic acid are two of the fruit's many bioactive phenolic compounds **[3-5]**. For phytochemical assessment, quality assurance, and standardisation of Karonda fruit and its derived products, accurate estimation of these compounds is crucial **[6-7]**. For the quantitative measurement of phenolic acids in intricate plant matrices, high-performance liquid chromatography (HPLC) has become the most popular and dependable analytical method **[8-10]**. The reported HPLC method development strategies, chromatographic conditions, sample preparation methods, validation parameters, and applications for the estimation of gallic acid and chlorogenic acid in Karonda fruit are compiled in this review. The purpose of this article is to provide researchers conducting analytical and phytochemical studies of Karonda with a thorough reference **[1-6]**.

Keywords: Karonda, *Carrisa carandas*, Chlorogenic acid, Gallic acid, HPLC, Method development, Phenolic acids

INTRODUCTION

The antioxidant and medicinal qualities of plants are largely attributed to phenolic compounds, a significant class of secondary metabolites **[11]**. Due to their widespread presence in fruits and medicinal plants as well as their established health benefits, chlorogenic acid and gallic acid have drawn a lot of attention **[12-13]**. The underutilised fruit crop Karonda (*Carissa carandas L.*) which is a member of the Apocynaceae

family, has long been utilised in Southeast Asian and Indian medicine **[14]**. The plant, which thrives in arid and semi-arid climates, is commonly found in parts of Africa, Southeast Asia, and India **[15]**. It yields tiny, berry-like fruits that are high in vital nutrients like dietary fibre, vitamins, and minerals **[16]**. The bioactivity of Karonda fruits is greatly enhanced by their high concentration of polyphenolic compounds, such as flavonoids and phenolic acids **[17]**. Numerous studies have suggested that these substances may provide protection against oxidative stress, which is linked to the aetiology of many chronic illnesses, such as cancer, diabetes, and cardiovascular disorders **[11, 18]**. The fruit, which has a strong antioxidant potential, can be eaten raw or processed into pickles, jams, and drinks **[16, 17]**. According to scientific research, the two main phenolic components of Karonda fruit are gallic acid and chlorogenic acid. Accurate estimation of these compounds requires the use of trustworthy analytical techniques. High-performance liquid chromatography (HPLC) is favoured over other analytical methods because of its sensitivity, specificity, and repeatability. The development of HPLC-based methods for estimating gallic acid and chlorogenic acid in Karonda fruit is the main aim of this review. The Carissa species has been widely used to treat a wide range of illnesses. Acidity, sores, flatulence, and indigestion are all signs of a weakened immune system. Diarrhoea, stomach problems, anorexia, intermittent fever, mouth ulcers, sore throat, syphilitic discomfort, burning sensations, scabies, and epilepsy are some of the human ailments. Vitamin C, dietary fibre, carbohydrates, lipids, proteins, and microelements are all abundant in Carissa fruits. Traditionally, the majority of Carissa species have been used to treat a wide range of illnesses, including headaches, chest pain, rheumatism, gonorrhoea, stomach pain, syphilis, hepatitis, oedema, rabies, asthma, and heart conditions.The fruit significantly reduced blood sugar levels in diabetic rats, according to a 2013 study published in the Journal of Ethnopharmacology. According to a different study that was published in the same journal in 2014, karonda extract improved the liver function of rats.

KARONDA (*CARISSACARANDAS*) FRUIT: BOTANICAL AND PHYTOCHEMICAL OVERVIEW

Karonda is a thorny shrub or small tree widely grown in India. The fruit is berry-like, initially green and turning reddish-purple upon ripening.

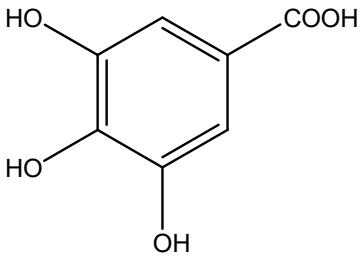
Phytochemical investigations of Karonda fruit have revealed the presence of,

- Phenolic acids
- Flavonoids
- Tannins
- Organic acids

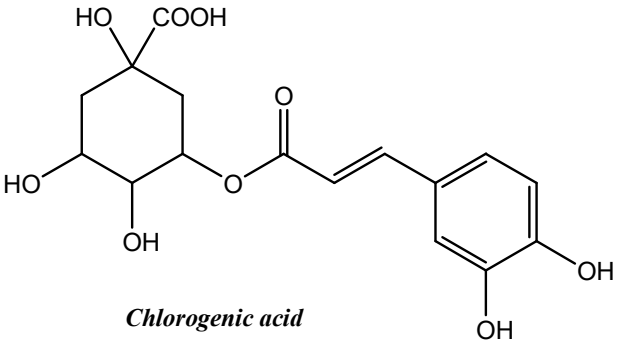
- Vitamins and minerals

Among the phenolic acids, chlorogenic acid and gallic acid contribute significantly to the fruit's antioxidant activity and medicinal value.

Chemical characteristics



Gallic acid OR 3,4,5-trihydroxybenzoic acid



Chlorogenic acid

Chlorogenic acid

- Ester of caffeic acid and quinic acid
- Molecular weight: 354.31 g/mol
- Highly polar
- UV absorption maximum around 325 nm

Gallic acid

Trihydroxy benzoic acid

- Molecular weight: 170.12 g/mol
- Strong UV absorption at 270–280 nm
- Their polarity and UV absorption properties make them suitable for analysis using reverse-phase HPLC with UV or diode array detection.

IMPORTANCE OF HPLC IN ESTIMATION OF PHENOLIC ACIDS:

One of the most popular analytical methods for determining the amount of phenolic acids in plant matrices, such as gallic acid and chlorogenic acid, is high-performance liquid chromatography (HPLC) **[24]**. Efficient separation and precise quantification of structurally similar phenolic compounds are made possible by the technique's high resolution and sensitivity **[25]**. When examining complex plant materials, like the fruit of the Karonda (*Carissa carandas),* which contains a variety of interfering substances like sugars, organic acids, pigments, and other secondary metabolites, this is especially crucial **[26]**. HPLC reduces analysis time and solvent consumption by enabling the simultaneous estimation of several phenolic compounds within a single analytical run **[27]**. Additionally, the technique is very accurate and repeatable, which makes it appropriate for regular quality control and standardisation research **[28]**.

Because it works well with polar compounds and aqueous mobile phases,

reverse-phase HPLC (RP-HPLC) with C_{18} columns is the most widely used chromatographic mode for phenolic acid analysis **[29]**. As a result, RP-HPLC is now the recommended technique for determining the amount of gallic acid and chlorogenic acid in Karonda fruit.

HPLC METHOD DEVELOPMENT FOR KARONDA FRUIT

Sample preparation

In order to accurately estimate the phenolic acids from Karonda fruit, sample preparation is essential. To minimise matrix interference and maximise the recovery of gallic acid and chlorogenic acid, efficient extraction is necessary. For phenolic acid analysis, a variety of extraction methods have been documented in the literature. The most widely used solvents are methanol or ethanol–water mixtures because of their capacity to dissolve polar phenolic compounds.

Because they shorten extraction times and increase extraction efficiency, advanced extraction methods like ultrasonication-assisted extraction have become more and more popular **[30, 31]**. When thorough extraction is necessary, conventional techniques like Soxhlet extraction are also used **[31]**. Prior to analysis, the samples are usually filtered through 0.45 µm membrane filters to eliminate particulate matter and safeguard the HPLC column **[32]**.

CHROMATOGRAPHIC CONDITIONS

Column selection

The choice of an appropriate chromatographic column is an important aspect in the achievement of effective separation **[33]**. The majority of the reported methods employ a C18 reverse-phase column with a length of 150 to 250 mm and a particle size of 3-5 µm **[33, 34]**. The columns are capable of separating chlorogenic acid and gallic acid effectively **[34]**.

Mobile phase

The mobile phase is normally composed of an organic solvent like methanol or acetonitrile and an aqueous phase **[33]**. The aqueous phase is normally acidified with 0.1% formic acid, acetic acid, or phosphoric acid to ensure a low pH **[34, 35]**. The low pH inhibits the ionization of phenolic acids, hence improving the resolution and reproducibility **[35]**.

Elution mode

Isocratic and gradient elution modes are used depending on the intended analysis **[33, 36]**. Isocratic elution is preferred for the routine analysis of chlorogenic acid and gallic acid because of its simplicity and ease of reproducibility **[33]**. Gradient elution is preferred when multiple phenolic compounds are to be analyzed together because of its ability to improve separation efficiency and prevent co-elution **[36]**.

Detection

Ultraviolet (UV) detection is a common method for the analysis of phenolic acids because of the high UV-absorbing properties of these compounds **[34]**. The detection of gallic acid is usually performed at wavelengths between 270 and 280 nm, while chlorogenic acid has a maximum absorption at wavelengths between 320 and 330 nm **[34, 37]**. The application of diode array detectors (DAD) enables the detection of several wavelengths simultaneously **[33]**.

Flow rate and injection volume

The optimized flow rates are typically in the range of 0.8-1.2 mL/min, which is a compromise between resolution and analysis time **[33]**. The injection volume of 10-20 µL is usually used to avoid peak broadening and column overload, while maintaining sufficient sensitivity **[33]**.

Linearity

Linearity is tested by preparing calibration curves using standard solutions of chlorogenic acid and gallic acid over an appropriate concentration range **[38]**. A good linear correlation between concentration and peak area is expected, which confirms the applicability of the method for quantitative analysis.

Precision

Precision is determined by intra-day and inter-day experiments, and the results are expressed as percentage relative standard deviation (%RSD) **[38]**. Typically, values below 2% confirm the high precision of the developed HPLC methods.

Accuracy

Accuracy is established by recovery experiments using the standard addition technique. Recovery within 95-105% establishes the accuracy and validity of the analytical method **[38]**.

Limit of detection & Limit of quantification

Low values of LOD and LOQ establish the high sensitivity of the HPLC methods, which can detect and quantify phenolic acids in low concentrations in Karonda fruit extracts **[38-39]**.

Robustness

Robustness is established by experiments that intentionally vary the chromatographic conditions such as flow rate, mobile phase, and wavelength **[38]**. Small variations in the results establish the robustness of the analytical methods.

APPLICATIONS OF HPLC ESTIMATION IN KARONDA FRUIT

There are many uses for HPLC-based gallic acid and chlorogenic acid estimation in Karonda fruit analysis **[40-41]**. These include the creation of nutraceutical and functional food products, standardization of herbal formulations, and quality control of raw fruit ingredients **[41-42]**. Additionally, quantitative analysis of phenolic acids supports antioxidant, pharmacological, and stability studies, thereby validating the

therapeutic potential of Karonda fruit **[40-43]**.

CHALLENGES AND FUTURE PERSPECTIVES

Despite its benefits, HPLC analysis of Karonda fruit has drawbacks, such as matrix interference from organic acids and sugars and phenolic compound co-elution **[44-45]**. For increased sensitivity and selectivity, future studies should concentrate on the creation of sophisticated analytical methods like HPLC-MS/MS and ultra-high-performance liquid chromatography (UHPLC) **[45-46]**. Promising avenues for future research include the use of green solvent-based extraction techniques and comparative analyses assessing the phenolic content of Karonda fruit at various stages of maturity **[47]**.

We review some of the parameters related to gallic acid and chlorogenic acid extraction and they are given in the following **Table 1 & 2**.

Table 1: Parameters of Gallic acid Extraction from Various Plants& Tablet

Plant	Plant part	Extraction method and solvent	HPLC Column	Retention time	Peakarea/Concentration	Analysed Compound
Terminalabellerica	Fruit	Soxhlet& Chloroform → Methanol → Ethanol	Phenomenex Luna 250 × 4.6 mm, 5 µm	3.60	3,190.82	Methanolic&ethanolic extracts analyzed
Woodfordiafruticosa	Flower	Soxhlet (hot percolation)&Methanol : Water (7:3)	RP-C18 250 × 4.6 mm, 5 µm	3.36	152,705	Hydro-alcoholic extract (WFF)
Cassia hirsuta	Seeds	Soxhlet (successive)&Petroleum ether → Chloroform → Ethanol	Enable C18G 250 × 4.6 mm, 5 µm	2.63 ± 0.002	Conc.-dependent	Ethanol extract used for GA quantification
Panicgranatum	Peel	Batch stirred extraction& Methanol	RP-C18 250 × 4.6 mm, 5 µm	3.41	Cultivar-dependent	Methanolic peel extract analyzed
Qualeagrandiflora / Q. parviflora	Stem bark	Exhaustive maceration& Hydro-alcoholic	Dionex Acclaim® 120 C18 250 × 4.6 mm, 5 µm	8.00	Regression-based	QG and QP extracts
Trigasornmas(poly herbal)	Tablet	Homogenization + stirring& Methanol	Zorbax SB C18 150 × 4.6 mm, 5 µm	3.42	23,860 – 6,654,917	Rat plasma bioanalysis
Mango cultivars	Peel & Seed	Reflux extraction& Hydro-alcoholic	Shim-pack C18 250 × 4.6 mm, 5 µm	3.41	Cultivar-dependent	Peel and seed extracts

Table 2: Parameters of Chlorogenic acid Extraction from Various Plants& Tablet

Sample matrix	Extraction conditions	HPLC Columns	Mobile Phase	Flow rate	Detection	Retention time	CGA Content
Viburnum tinus	Methanol; maceration at room temperature for 8 h.	C-18	0.2% Phosporic acid(A) :Aceto nitrile(B)	1.2	330	7.68	0.014-0.507%W/W
Roasted coffee	Soxhlet extraction; methanol:water (50:50).	C-18	Water +1%Phosporic acid(A):Aceto nitrile(B)	1.0	324	~7.0	Total CGA:2.6g/100g
Coffeearabica extract	Extract (regional coffee samples)	C-18	KH2PO4:Aceto nitrile	1.0	274	Not reported	22-25%W/W
Green Coffee beans	Hot and Cold extraction: water,methanol,propanol	C-18	Not specified	Not reported	Not reported	7.2(5-CQA)	Max yield 11% (hot water)
Coffee leaves	Methanol:water(70:30) 70c repeated extraction	C-18	Not specified	Not reported	Not reported	Not reported	CGA quantified:stable and reproducible

CONCLUSION

The fruit of the Karonda (Carissa carandas) is a valuable natural source of gallic and chlorogenic acids. The best analytical method for developing and validating procedures for the precise measurement of these phenolic acids has been shown to be HPLC. Reliable quantification is ensured by appropriate chromatographic condition optimisation and adherence to validation guidelines, supporting the standardisation, quality control, and scientific application of Karonda fruit in the food and pharmaceutical industries.

REFERENCES

[1] Nadkarni KM. *Indian Materia Medica*, Vol 1. 3rd ed. Mumbai: Popular Prakashan; 2007. p. 292-295.

[2] Kirtikar KR, Basu BD. *Indian Medicinal Plants*, Vol 2. 2nd ed. Dehradun: International Book Distributors; 2005. p. 1548-1550.

[3] Verma RP, Rani A. Phytochemical constituents of *Carissa carandas* and their pharmacological significance: A review. Int J Pharm Sci Rev Res 2013; 20: 30-34.

[4] Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci 1997; 2: 152-159.

[5] Santana-Galvez J, Cisneros-Zevallos L, Jacobo-Velazquez DA. Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. Molecules 2017; 22: 358.

[6] Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman & Hall; 1998.

[7] ICH. Validation of analytical procedures: Text and methodology Q2(R1). International Conference on Harmonisation; 2005.

[8] Snyder LR, Kirkland JJ, Dolan JW. *Introduction to Modern Liquid Chromatography*. 3rd ed. New York: Wiley; 2010.

[9] Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci 2007; 30: 3268-3295.

[10] Robards K, Antolovich M. Analytical chemistry of fruit bioflavonoids: A review. Analyst 1997; 122: 11R-34R.

[11] Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties**.** Molecules. 2010;15(10):7313-7352.

[12] Farah A, Donangelo CM. Phenolic compounds in coffee. Braz J Plant Physiol. 2006;18(1):23-36.

[13] Khadem S, Marles RJ. Monocyclic phenolic acids; hydroxy- and polyhydroxybenzoic acids: occurrence and recent bioactivity studies. **Molecules**. 2010;15(11):7985-8005.

[14] Kaunda JS, Zhang YJ. The genus *Carissa*: an ethnopharmacological, phytochemical and pharmacological review. Nat Prod Bioprospect**.** 2017;7:181-199.

[15] Nadkarni KM. *Indian MateriaMedica*. 3rd ed. Mumbai: Popular Prakashan; 2007.

[16] Azeez SH, Karunakaran G, Tripathi PC, Shivashankara KS. Nutritional and antioxidant properties of karonda (*Carissa carandas* L.) fruits. Indian J Agric Sci**.** 2016;86(6):784-789.

[17] Pandey A, Tripathi S. Concept of standardization, extraction and pre-phytochemical screening strategies for herbal drug. J PharmacognPhytochem**.** 2014;2(5):115-119.

[18] Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am J ClinNutr**.** 2005;81(1):215S-217S.

[19] Saeed W, et al. Phytochemical profiling and antioxidant activity of *Carissa carandas* fruit extracts. Antioxidants**.** 2024;13(9):1037.

[20] Snyder LR, Kirkland JJ, Dolan JW. *Introduction to Modern Liquid Chromatography*. 3rd ed. New Jersey: Wiley; 2010.

[21] Warrier PK, Nambiar VPK, Ramankutty C. *Indian Medicinal Plants*. Vol 1. Hyderabad: Orient Longman; 2004.

[22] Pandey SK, Patel AK, Verma P, Verma R. Antidiabetic activity of *Carissa carandas* Linn. fruit in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2013;147(3):674-678.

[23] Pandey SK, Patel AK, Verma P, Verma R. Hepato-protective activity of *Carissa carandas* Linn. fruit against liver damage in rats. J Ethnopharmacol**.** 2014;151(2):889-894.

[24] Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem. 2003;51(10):2866–87.

[25] Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci. 2007;30(18):3268–95.

[26] Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313–52.

[27] Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A. 2004;1054(1–2):95–111.

[28] Ignat I, Volf I, Popa VI. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem. 2011;126(4):1821–35.

[29] Crozier A, Jaganath IB, Clifford MN. Phenols, polyphenols and tannins: an overview. Nat Prod Rep. 2009;26(8):1001–43.

[30] Dent M, Dragović-Uzelac V, Penić M, Bosiljkov T, Levaj B. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols. Food TechnolBiotechnol. 2013;51(1):84-91.

[31] Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci. 2007;30(18):3268-3295.

[32] Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 6th ed. Belmont (CA): Thomson Brooks/Cole; 2007.

[33] Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd ed. Hoboken (NJ): John Wiley & Sons; 2010.

[34] Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules. 2013;18(2):2328-2375.

[35] Kazakevich Y, Lobrutto R. HPLC for pharmaceutical scientists. Hoboken (NJ): John Wiley & Sons; 2007.

[36] Snyder LR, Dolan JW. High-performance gradient elution: the practical application of the linear-solvent-strength model. Hoboken (NJ): John Wiley & Sons; 2007.

[37] Ignat I, Volf I, Popa VI. A critical review of methods for characterization of polyphenolic compounds. Food Chem. 2011;126(4):1821-1835.

[38] International Conference on Harmonisation (ICH). ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.

[39] Shrivastava A, Gupta VB. Methods for determination of limit of detection and limit of quantitation of analytical methods. Chron Young Sci. 2011;2(1):21-25.

[40] Ignat I, Volf I, Popa VI. A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chem. 2011;126(4):1821-1835.

[41] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270-278.

[42] Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines – A review. Int J BiodiversConserv. 2012;4(3):101-112.

[43] Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci. 2007;30(18):3268-3295.

[44] Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd ed. Hoboken (NJ): John Wiley & Sons; 2010.

[45] Ribani M, Bottoli CBG, Collins CH, Jardim ICSF, Melo LFC. Validation for chromatographic and electrophoretic methods. Quim Nova. 2004;27(5):771-780.

[46] Swartz ME. UPLC™: An introduction and review. J LiqChromatogrRelat Technol. 2005;28(7-8):1253-1263.

[47] Chemat F, Vian MA, Cravotto G. Green extraction of natural products: Concept and principles. Int J Mol Sci. 2012;13(7):8615-8627.