Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 45 No. 4 (2024)

Evaluation of Profiling of Total Phenolics and Flavonoids of Carissa Carnadas L. Fruits and Leaves on LC-HRMS

Meghna Choudhary [1], Amit Kumar [2*], Nupur Rani Agarwal [3*], Ashok Singh [4], Jayesh Yadav [5], Mani Ratnam [6]

[1] *Shri Venkateshwara University, Amroha, Uttar Pradesh, India*

[2] *Assistant Professor, Organic Chemistry, Shri Venkateshwara University, Amroha, Uttar Pradesh, India*

[3] *All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi, India*

[4] *National Dope Testing Laboratory, New Delhi, Delhi*

[5] *Singhania University, Rajasthan, India*

[6] *Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India*

Abstract:- *Craissa Carnadas L.* (Karonda) is hard, drought tolerant berries.There is potential for development and promotion in the wastelands of India." The fruits are astringent as it contains high content of pectin which is use as a preserves and leaves have medicinal values such as pain relief, gut heath, inflammation, stomach ailments and diarrhea. In this study, the total phenolics and flavonoids are analysed on LC-HRMS orbitrap 240. The photochemical profile of the individual phenolic components includes caffeic acid, 2,4-dihydroxybenzoic acid (or gentisic acid), chlorogenic acid, ferulic acid, gallic acid, o-coumaric acid, p-hydroxybenzoic acid (or salicylic acid), protocatechuic acid, syringic acid, trans-cinnamic acid, and vanillic acid. The flavonoids include apigenin, catechin, hesperetin, kaempferol, luteolin, myricetin, naringenin, quercetin, rutin, and umbelliferone. The qualitative and quantitative analysis was performed on LC-HRMS. For Quantitative analysis “Furosemide-D5”, is used as an internal standard in the negative mode analysis. In this study, Carissa carandas (karonda) fruits and leaves are rich in secondary metabolites, particularly phenols and flavonoids. Four samples, including fresh and dry fruits and leaves, were prepared using two extraction solvents: pure methanol and methanol with hydrochloric acid (99:1). The methanol extract of fresh fruits showed the highest concentrations of phenolics, such as caffeic acid, chlorogenic acid, and ferulic acid, while fresh leaves had higher levels of protocatechuic acid. Flavonoid concentrations were also highest in fresh fruits, particularly for myricetin, rutin, and quercetin. Drying influenced the phenolic and flavonoid composition, with significant variations across samples.

Keywords*: LC-HRMS, karonda, fruits, leaves, phenolics, flavonoids.*

1. Introduction

Karonda (Carissa carandas L.), an evergreen shrub from the Apocynaceae family, has economic, commercial, medicinal, and nutritional value. This hardy, drought-tolerant plant thrives in dryland conditions and adapts well to diverse soils and varying climatic changes. (1).The plant provides vital support to tribal communities across various regions of India, including Bihar, Dehradun, Jhansi, Rajasthan, West Bengal, Uttar Pradesh, and the southern parts of the country. (2).Karonda, also called Crane Berry in English, "Karonda in Devanagari and Hindi, "Karamcha" in Bengali, and "Ci-Huang-Guo" in Chinese, is found in tropical and subtropical regions of countries like Pakistan, India, Sri Lanka, Myanmar, Bangladesh, and Nepal. (3). A plant with rhombus-shaped, conical leaves bears delicate white flowers. Its fruit grows in clusters of 3–10, resembling tiny berries. The arrangement and shape of its elements evoke the image of a human eye, with the clustered fruits forming the iris and the flowers

as highlights (4). The unripe fruits of karonda are pinkish-white, turning red or dark purplish when fully ripe. The fresh juice from these ripe fruits is rich in vitamin C and iron, making them effective in combating scurvy and iron deficiency. Similar fruits like acerola ("Barbados cherry") and jambul are also valued for boosting iron levels and preventing vitamin C deficiency. (5).

The berry-sized fruits of *Carissa carandas L.* are visually attractive and commonly used as additives in various products-condiments, pickles, and spices in India.Rich in macro- and micronutrients, organic acids, and pectin, karonda fruits are ideal for producing beverages, jams, jellies, and curries. Their versatility in culinary applications, coupled with their impressive nutritional profile, makes **C. carandas** a valuable ingredient in both traditional and modern food products. (6). *Carissa carandas L.* (karonda) fruits are known for their taste. Karonda fruits are sour and astringent, transitioning from acidic too sweet as they ripen. Due to their high pectin content, they are not typically consumed fresh but are popular in the processing industry for making preserves. Rich in iron and vitamin C, they offer antiscorbutic benefits and help prevent anemia. Additionally, karonda is used to alleviate stomach aches, act as an anthelmintic, and treat various ailments. (7).

In Ayurveda, unripe karonda fruits are utilized as an astringent, appetizer, antipyretic, and antidiabetic. Traditional healers in Kodagu, Karnataka, also incorporate the fruit into tribal medicine. Although literature on karonda is limited, its proximate composition and medicinal significance are widely acknowledged. (8-9).

*Carissa carandas L.(*karonda) is traditionally valued for its extensive medicinal properties. It is reported for the treat of various health issues. Karonda is known to help with issues such as upset stomach and neurological impairments, and it also possesses cardiotonic, anthelmintic, and antihypertensive effects. (10,11). The fruits are known to help with conditions such as piles, appetite loss and nervous disorders, while also addressing Karonda is used to address various health issues, including edema, colic, splenomegaly, hepatomegaly, amenorrhea, cardiovascular diseases, brain anorexia, and fever. (11-13).

In addition, *C. carandas* is also, used for the treatment of various diseases which is utilized in traditional medicine for treating a variety of conditions, including epilepsy, diarrhea, dog bites, myopathic spasms, coughs, colds, leprosy, itching, inflammation, malaria, and skin infections. (5,6,13,15). Karonda is believed to restore female libido, improve liver function, and combat microbial infections and intestinal worms. Additionally, it may help counteract blood putrefaction and alleviate rheumatoid arthritis. (references (5,15).

The leaves of karonda are reported to be effective in treating various health conditions, including diarrhea, fever, earaches, syphilis-related pain, and snake bites. (12,13). Meanwhile, the roots of karonda are said to enhance digestion and treat various conditions, including scabies, intestinal worms, stomach disorders, diabetes, pruritus, ulcers, and hypertension. (14). This wide range of applications underscores the significance of karonda in traditional healing practices.

C. carandas is an underutilized Nutritional Powerhouse and an often-overlooked berry fruit, is rich in essential micronutrients like zinc, copper, and manganese, as well as macro-nutrients including carbohydrates, protein, fat, and fibre (6) Nutritional deficiencies in Asia, particularly in iron and zinc, pose significant public health challenges. Alarmingly, nearly half of pregnant women and 40% of preschoolers in Southeast Asia are affected by iron deficiency anemia, while over 30% of the population suffers from zinc deficiency (16-19). Despite the availability of approximately 5,538 food crops globally, 12 plants and 5 animal species account for 75% of the world's food production, with just 3 plant species providing more than half of the food energy needs. This lack of crop diversity contributes to food insecurity.

The Role of Crop Diversity introducing neglected and underutilized crops like *C. carandas* into agricultural systems can enhance food diversity, offering more options for farming communities to meet evolving household and market needs. This approach has the potential to reduce nutrient deficiencies and strengthen local food systems, especially in smallholder contexts, which are crucial for sustainable global food security.

Medicinal Potential in *C. carandas* species is not only nutritionally beneficial, it is also boasting traditional medicinal claims backed by pharmacological evidence. Its extracts whether Methanol, ethanol, and water extracts of **C. carandas** exhibit biological potentials comparable to synthetic drugs. Moreover, this plant presents

promising opportunities for sustainable agriculture, potentially boosting local economies and providing farmers with a new source of income. This can drive further research and investment in its production and commercialization. The versatility of C. carandas extends to its potential for developing functional foods, natural preservatives, and innovative products, thanks to its impressive antioxidant, antimicrobial, anti-tyrosinase, and anti-inflammatory properties. With its promise for sustainable harvesting, C. carandas represents a significant step toward addressing nutritional deficiencies and food security challenges. (20).

2. Material and Methods

2.1. Chemicals and standards

Furosemide-D5 was used as Internal Standard of clearsynth, Mumbai, Maharashtra for phenolics and flavonoids study. Methanol is used for extraction were provided from Thermofisher, Belgium and Hexane from Merck, Germany.

2.2. Plant material

Fruits and Leaves samples of *C. carandas* collected from the northeastern part of India, Faridabad and authenticated by Prof. G. Sudarsanam, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The fruits and leaves were collected and half of the fruits and leaves are kept for drying at room temperature for 7 to 8 months and air dried in shed. The information of the plant was recorded, the higher temperature was 34°C and lower temperature was 26°C, location have a Latitude 28.4089°N and Longitude 77.4089°E. The collection of plant was August,2022 and the climatic was humid.

2.3. Extraction of fruits and leaves of sample preparation

The fresh fruits and leaves and dried fruits and leaves are extracted in the motor pistil. Total phenolics and flavonoids are extracted in two different extraction protocol. The first method of extraction was extracted in pure methanol. The dried fruit and leaves were weighed on analytical balance 0.5 g in 2.5 ml in methanol. And fresh fruits and leaves are weighed in 2g into 10 ml. After 45 mins of extraction in the motor pistil, transfer in the glass screw tube and shake for 30 mins in the shaker and centrifuge for 20 mins at 5000 RPM. Transfer the upper layer of the methanolic extraction in other glass screw tube (1ml) and keep for drying in nitrogen evaporator at 60º C at flow of 1.5 min/ml. after the methanolic extract was dried add recon solution of 90:10 in the glass tubes. Four samples were injected with analysis for quantification and four samples are injected with Internal Standard D5-Furosemide for qualification. The internal standard was added in mobile phase at the concentration of 25 ng/ml. Add 300 ul of recon solvent in the glass tubes. Vortex the samples for 10 mins on the vortex shaker at 2500 RPM. Transfer in the Eppendorf and centrifuge for 5 mins at 10,000 RPM and transfer 150 ul in plastic vials. Injects the samples on LC-HRMS Orbitrap 240. The second protocol is same but the extraction was with methanol:HCl (99:1). The mobile phase consists of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B).

2.4. LC-HRMS analysis

100 µl from each sample was transferred into plastic vials. The 5ul of samples was injected in LC-HRMS with the parameters. The phenolics and flavonoids compounds were identified on freestyle on the basis of parent (m/z) and identify the retention time of each compound of phenolics and flavonoids. The database is prepared on the parent mass in negative mode ionization. The data is analyzed on Trace finder software.

2.5. Determination of Phenolics and Flavonoids

LC-HRMS Conditions

The mobile phase consisted of an aqueous phase of 0.1% formic acid in water (A) and an organic phase of 0.1% formic acid in methanol (B). The gradient conditions are detailed in Table 1.

The flow rate was set at 0.25 mL/min, and the analytical column used was a 2.1 x 50 mm UPLC BEH C18 column with 1.7 µm particles (Waters, USA). The column temperature was maintained at 40º C, and the sample injection volume was 5 µL for both phenolics and flavonoids. The metabolites were eluted and monitored using a UPLC

system, which pumped directly to an Orbitrap mass spectrometer (240). The data were optimized for the analysis of phenolics and flavonoids.

The data analysis for quantifying and qualifying phenolics and flavonoids was conducted using Trace Finder software.

The samples were analyzed in Full Scan Mode, with a scan range of m/z 50-750. The Orbitrap resolution was set at 45,000, and the total run time was 20 minutes. The parameters for the ion sources are detailed in Table 2.

Table 1: The solvent gradient in the method for the separation of phenolics and flavonoids.

S.NO.	Time	Flow [ml/min]	Solvent A (%)	Solvent B (%)	Curve
1.	0.000	0.250	100.0	0.0	5
2.	2.000	0.250	90.0	10.0	5
3.	6.000	0.250	70.0	30.0	5
4.	8.000	0.250	50.0	50.0	5
5.	10.000	0.250	30.0	70.0	5
6.	12.000	0.250	10.0	90.0	5
7.	14.000	0.250	0.0	100.0	5
8.	15.000	0.250	0.0	100.0	5
9.	16.000	0.250	100.0	0.0	5
10.	20.000	0.250	100.0	0.0	5
Total Time	20.00 Stop Run				

Table 2: Parameters of Ion Source

S.NO.	Properties	Parameters
1.	Ion source Type	H-ESI
2.	Spray Voltage	Static
3.	Positive Voltage(v)	4000
4.	Negative Voltage (V)	3000
5.	Gas Mode	Static
6.	Sheath Gas (Arb)	60
7.	Aux Gas (Arb)	10
8.	Sweep Gas (Arb)	10
9.	Ion Transfer Tube Temp (° C)	320
10.	Vaporizer Temp (° C)	400

2.6 Data Analysis

The quantification of phenolic compounds analysed includes caffeic acid, 2,4-dihydroxybenzoic acid, chlorogenic acid, ferulic acid, gallic acid, o-coumaric acid, p-coumaric acid, p-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, and trans-cinnamic acid, which are present in dry leaves, fruits, and fresh fruits and leaves. On the other hand, the flavonoids analysed include apigenin, catechin, hesperetin, kaempferol, luteolin, myricetin, naringenin, quercetin, rutin, and umbelliferone.

Total phenols and flavonoids are quantified with internal standard at different retention time and all the observation is in negative mode. The database details are mention (Table 3) for phenolics and (Table 4) for flavonoids.

The scan window range in full scan is 50.0000-750.0000. An internal standard(D5-Furosemide) molecular mass is 334.0318 and used in a negative mode ionization. The collision energy is 30kv. The quantification of phenols and flavonoids were performed in triplicates. The database was prepared in the trace finder software to identify the retention time. The retention time analysed of an internal standard is "9.97"

Table 3: Database details of phenolic acid.

S.NO.	COMPOUND	MOLECULAR MASS	PARENT M/Z	RT	COLLISION ENERGY	ION MODE
1.	Caffeic acid	180	179.0349	6.59	30	ES^-
2.	Chlorogenic acid	354	353.0878	6.09	30	ES^-
3.	Ferulic acid	194	193.0506	8.44	30	ES^-
4.	Gallic acid	170	169.0143	6.85	30	ES^-
5.	Gentisic acid or 2,4-dihydroxy benzoic acid	154	153.0193	4.37	30	ES^-
6.	o-coumaric acid	164	163.0401	8.01	30	ES^-
7.	p-hydroxybenzoic acid or salicyclic acid	164	137.0244	9.57	30	ES^-
8.	Protocatechuic acid	154	153.0193	5.59	30	ES^-
9.	Syringic acid	198	197.0456	7.12	30	ES^-
10.	Trans-cinnamic acid	148	147.0452	8.47	30	ES^-
11.	Vanillic acid	168	167.0398	8.62	30	ES^-

Table 4: Database Details of Flavonoids

S.NO.	COMPOUND	MOLECULAR MASS	PARENT M/Z	RT	COLLISION ENERGY	ION MODE
1.	Apigenin	270	269.0456	10.97	30	ES^-
2.	Catechin	290	289.0718	6.89	30	ES^-
3.	Hesperetin	302	301.0718	10.51	30	ES^-
4.	Kaempferol	286	285.0405	10.88	30	ES^-
5.	Luteolin	286	285.0405	10.87	30	ES^-
6.	Myricetin	318	317.0303	6.83	30	ES^-
7.	Naringenin	272	271.0162	10.48	30	ES^-
8.	Quercetin	302	301.0354	10.26	30	ES^-
9.	Rutin	610	609.1461	9.01	30	ES^-
10.	Umbelliferone	162.14	161.0244	9.32	30	ES^-

3. Results and Discussion

Carissa carandas (karonda) fruits and leaves are rich in secondary metabolites, particularly phenols and flavonoids. Therefore, all the phenols and flavonoids are potentially good as a source. The four different samples are prepared of fresh fruits and leaves and second is dry fruit and leaves. All the compounds of flavonoids and phenols were identified and quantify with an internal standard of negative ionization i.e. Furosemide-D5. The samples are prepared in two different extraction solvent-pure methanol and methanol and hydro chloroacetic acid (99:1). The methanol extract of fresh **Carissa carandas** fruits exhibits the highest concentration of phenolic compounds, including caffeic acid, chlorogenic acid, ferulic acid, Gentisic acid (2,4-dihydroxybenzoic acid), o-coumaric acid, p-hydroxybenzoic acid (salicylic acid), syringic acid, trans-cinnamic acid, vanillic acid, and gallic acid. However, the highest concentration Protocatechuic acid are observed in the methanol extract of fresh leaves. This indicates that the phenolic composition differs between the fruits and leaves, with specific compounds being more prevalent in each part. The concentration of Gentisic acid (2,4-dihydroxybenzoic acid) is significantly higher in fresh fruits, measuring 3.60×10^5 (360,000), compared to fresh leaves, dry leaves, and dry fruits, where it reaches its lowest concentration of 9.76×10^2 (976) in fresh leaves. This substantial discrepancy indicates a much greater distribution of Gentisic acid in fruits than in leaves. In contrast, the concentration of Protocatechuic acid is highest in dry leaves at 7.11×10^4 (71,100) and lowest in fresh leaves at 3.04×10^2 (304), suggesting that the drying process may concentrate or preserve Protocatechuic acid, resulting in a higher amount in dry leaves compared to fresh ones. Similarly, Chlorogenic acid is most abundant in fresh fruit, with a concentration of 7.77×10^5 (777,000), while the lowest concentration is found in dry fruit at 7.24×10^3 (7,240), indicating a significant decline in Chlorogenic acid levels after drying. Gallic acid also shows a peak concentration in fresh fruit at 3.70×10^4 (37,000) and a low point in fresh leaves at 3.64×10^1 (36.4), further demonstrating that this compound is predominantly concentrated in fresh fruits. Caffeic acid follows a similar trend, being highest in fresh fruit at 1.14×10^5 (114,000) and lowest in fresh leaves at 1.82×10^3 (1,820). Syringic acid also shows greater concentration in fresh fruit, with a value of 2.69×10^3 (2,690) compared to fresh leaves at 1.86×10^2 (186). The concentration of o-Coumaric acid is highest in fresh fruit at 2.29×10^5 (229,000) and lowest in fresh leaves at 1.70×10^3 (1,700), indicating a clear preference for accumulation in fruits. Moreover, Trans-cinnamic acid reaches its peak concentration in fresh fruit at 7.29×10^3 (7,290), with the lowest in fresh leaves at 5.29×10^1 (52.9). Ferulic acid shows the highest concentration in fresh fruit at 2.18×10^5 (218,000) and the lowest in fresh leaves at 1.31×10^3 (1,310). Both Vanillic acid and Salicylic acid are similarly concentrated in fresh fruit, with values of 5.65×10^4 (56,500) and the lowest in fresh leaves at 1.75×10^2 (175), highlighting a consistent trend of higher phenolic concentrations in fresh fruits compared to fresh leaves.

The methanol extract of fresh fruits demonstrates the highest concentrations of flavonoids, including myricetin, rutin, quercetin, hesperetin, kaempferol, and apigenin. In contrast, catechin, naringenin, and luteolin show the lowest concentrations in fresh leaves. This distribution indicates that while fresh fruits are rich in certain flavonoids, fresh leaves provide greater amounts of others, highlighting the variability in flavonoid content between different parts of the plant. Myricetin exhibits the highest concentration in fresh fruit, measuring 1.32×10^4 (13,200), while its lowest concentration is found in dry fruit at 5.84. Similarly, catechin shows the highest concentration in fresh leaves at 3.16×10^5 (316,000), with the lowest concentration in dry fruit at 5.94×10^2 (594). Rutin is highest in fresh fruit at 7.20×10^3 (7,200) and lowest in dry fruit at 4.54×10^1 (45.4). Quercetin also reaches its peak in fresh fruit at 2.00×10^5 (200,000) and is lowest in dry leaves at 2.43×10^3 (2,430). Naringenin shows its highest concentration in fresh fruit at 1.77×10^4 (17,700) and lowest at 4.71×10^2 (471). Hesperetin is most concentrated in fresh fruit at 4.44×10^3 (4,440), with its lowest level in dry leaves at 5.63. Kaempferol has the highest concentration in fresh fruit at 9.32×10^3 (9,320) and the lowest in fresh leaves at 2.69×10^2 (269). In contrast, luteolin exhibits the highest concentration in fresh leaves at 1.28×10^5 (128,000), while the lowest is found in dry fruit at 4.19×10^2 (419). Apigenin is highest in fresh fruit at 9.75×10^2 (975) and lowest in fresh leaves at 1.21×10^2 (121). Finally, umbelliferone has its highest concentration in fresh fruit at 1.24×10^3(1,240) and its lowest in dry fruit at 1.60×10^2(160). These findings indicate significant variations in flavonoid concentrations across different parts of the plant, with fresh fruits generally showing higher levels of certain compounds, while fresh leaves may have the highest concentrations for others.

In a comparative analysis, of phenolics of Methanol and HCl extracts of various acids across different plant parts, 2,4-dihydrobenzoic acid shows the highest concentration in dry fruit at 1.59E+05 and the lowest in fresh leaves at 7.76E+03. Protocatechuic acid is most abundant in fresh leaves at 1.10E+05 and least in dry fruit at 3.01E+03. Chlorogenic acid is highest in fresh leaves, with a concentration of 2.18E+05, and lowest in dry fruit at 6.07E+03. Caffeic acid is at its peak in dry leaves at 6.89E+04, with the lowest concentration found in fresh leaves at 1.54E+04. Gallic acid reaches its maximum in fresh fruit at 1.07E+04, while fresh leaves contain the least, at 1.09E+03. Syringic acid is most concentrated in dry leaves, at 7.84E+03, and lowest in dry fruit at 6.42E+02. O-coumaric acid is highest in fresh fruit, with a value of 7.74E+04, and lowest in dry fruit at 5.69E+02. Trans-cinnamic acid peaks in fresh fruit at 2.01E+03, with its lowest concentration in fresh leaves at 2.28E+00. Ferulic acid is most abundant in fresh fruit, at 5.85E+04, and least in fresh leaves, at 9.76E+03. Vanillic acid reaches its maximum in fresh fruit at 1.34E+04, while its lowest level is in dry fruit, at 5.22E+02. Finally, salicylic acid is highest in fresh fruit at 3.84E+04, with the lowest concentration found in dry fruit at 4.68E+03.

In a study examining the flavonoid content extracted using methanol and HCl, myricetin was found to have the highest concentration in fresh fruit at 4.42E+03 and the lowest in fresh leaves at 3.84E+01. Similarly, catechin reached its peak in fresh fruit at 3.89E+04, with the lowest concentration in fresh leaves at 3.97E+02. Rutin was most abundant in dry leaves at 2.69E+03, while the lowest level was in dry fruit at 5.44E+01. Quercetin showed the highest concentration in fresh fruit at 8.61E+04, and the lowest in dry fruit at 4.71E+03. Naringenin peaked in fresh leaves at 3.97E+04, while its lowest concentration was in dry leaves at 4.66E+02. Kaempferol was most concentrated in fresh leaves at 1.31E+04 and least in dry fruit at 7.27E+01. Hesperetin reached its maximum in fresh fruit at 1.67E+03, with the lowest levels in both dry fruit and fresh leaves at 1.90E+01. Luteolin was highest in fresh leaves at 7.07E+04 and lowest in dry fruit at 3.72E+02. Apigenin had its peak concentration in fresh fruit at 5.13E+02, with the lowest concentration in dry fruit at 3.48E+01. Finally, umbelliferone was highest in fresh fruit at 1.62E+03, with the lowest level in dry fruit at 8.02E+01.

4. Conclusion

Results reported here clearly highlighted that the phenolics and flavonoids variation based on LC-HRMS analysis is able to discriminate 11 compounds of phenolics and 10 compounds of flavonoids. The methanol extract of fresh leaves reveals a significant concentration of key phytochemicals, particularly phenolics and flavonoids. Among the phenolic compounds, chlorogenic acid is found in the highest concentration, demonstrating its prominence in fresh leaf extracts. On the other hand, within the flavonoid group, catechin is the most abundant, showcasing its dominance in the methanol extract of fresh leaves. These findings highlight the rich presence of chlorogenic acid and catechin in fresh leaves, which may contribute to their potential health benefits and pharmacological properties. In the methanol and HCl extract of fresh leaves, chlorogenic acid is found to be the phenolic compound present in the highest concentration, indicating its abundance in the leaves. Conversely, in the methanol and HCl extract of fresh fruit, quercetin stands out as the flavonoid with the highest concentration. This highlights a distinct distribution of bioactive compounds within the plant, with chlorogenic acid predominantly concentrated in the leaves and quercetin primarily found in the fruit. These variations suggest that different parts of the Carissa carandas plant may offer unique health benefits due to the presence of these key compounds.

Acknowledgements

We thank NDTL-National Dope Testing Laboratory, New Delhi for providing LC-HRMS facility.

Disclosure Statement

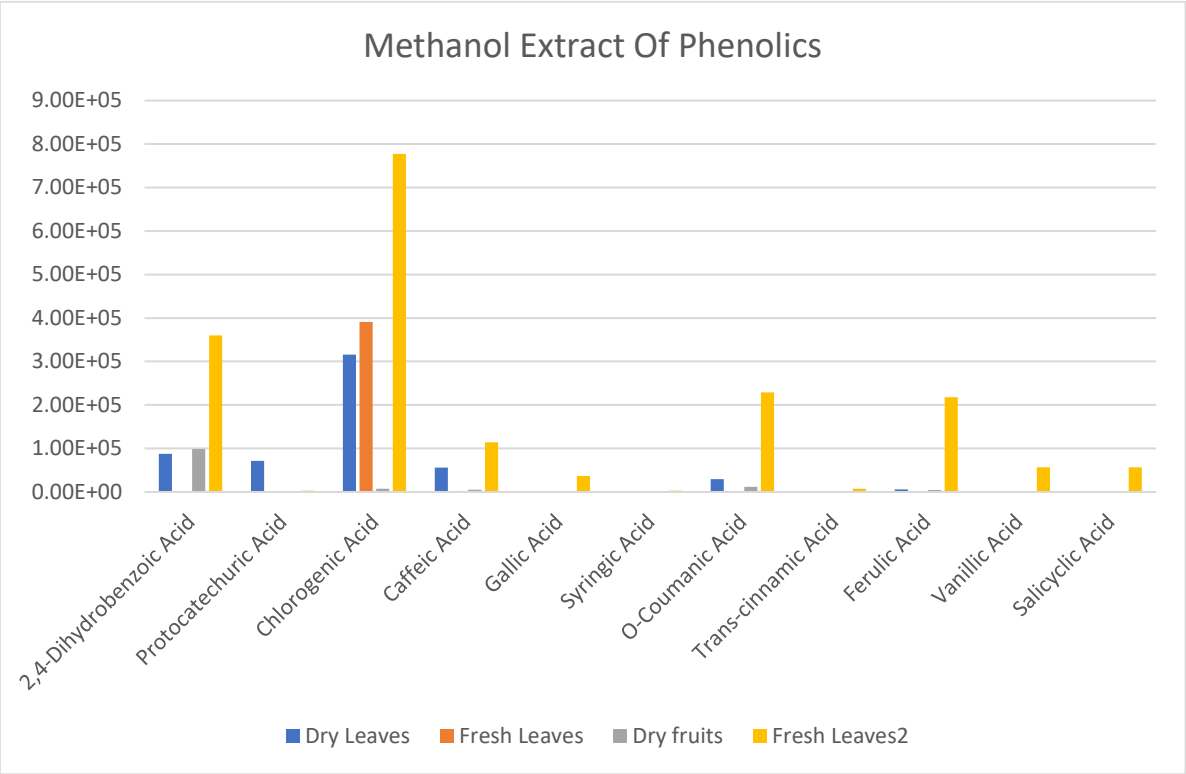
The authors declare that they have no competing interests.

Reference

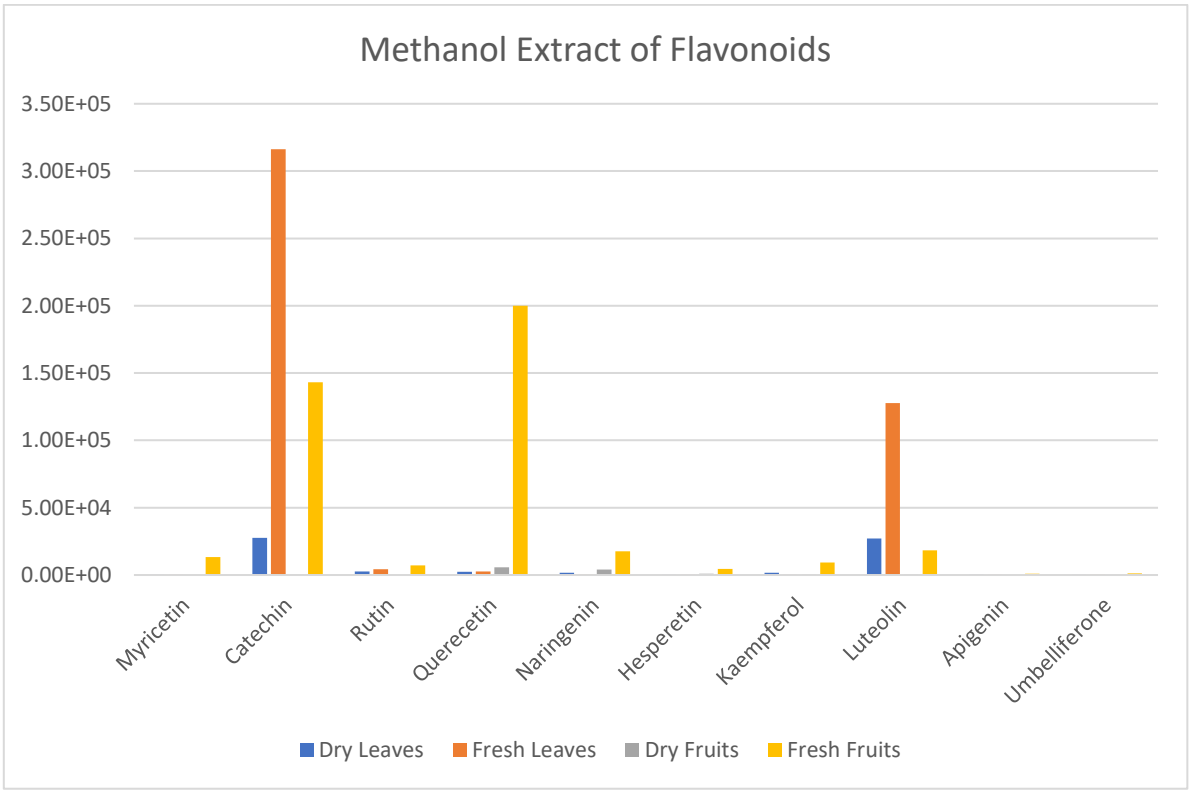
1. G. Thiyagarajan, S. Rajasekaran, S. Balamurugan, S. Karthikeyan, Studies on the effect of phytochemical and mineral analysis of kalakai (Carissa carandas L.) fruit, Int. J. Curr. Res. 12 (10) (2020) 14066–14072, https://doi.org/10.24941/ ijcr.39772.10.2020.

2. Banik B C, Ghosh S N and Singh S R. 2012. Research and development in karonda (Carissa carandas), a semi wild fruit in India. Proceedings of the Ist IS on Wild Relatives, Subtropical & Temperate Fruit & Nut Crops, Eds.: Aradhya M K and Kluepfel D A. Acta Horticulturae 948, ISHS 2012.
3. S. Begum, S.A. Syed, B.S. Siddiqui, S.A. Sattar, M.I. Choudhary Carandinol: first isohopane triterpene from the leaves of *Carissa carandas* L. and its cytotoxicity against cancer cell lines Phytochem. Lett., 6 (1) (2013), pp. 91-95, 10.1016/j.phytol.2012.11.005.
4. R.P. Dalal, N.A. Thakur, A. Singh Nutritional value of Karonda (*Carissa carandas* Linn). A non-conventional fruit under semi-arid condition of Punjab Indian J. Agrofor., 12 (2010), Article 102104.
5. M. Mahajan, H.K. Bons, G.K. Dhillon, P.A. Sachdeva Unlocking the impact of drying methods on quality attributes of an unexploited fruit,karonda (*Carissa carandas* L.): a step towards food and nutritional security.
6. K. Jayakumar, B. Muthuraman:Traditional uses and nutrient status of Indian native plant fruit (*Carissa carandas* Linn.) WSN., 96 (2018), pp. 217-224
7. Das S C, Prakash J, Deb A K and Biswas T. 2013. Medicinal value of underutilized fruits in hilly Tripura. Acta Horticulture 972:135–41.
8. Iyer C M and Dubhash P J. 2006. Anthocyanin of Karwand(Carrisa carandus) and studies on its stability in model systems.Journal of Food Science and Technology 30: 246–8.
9. Itankar P R, Lokhande P J, Verma Pr R, Arora S K, Sahu R A and Patil A T. 2011. Antidiabetic potential of unripe Carrisa carandas Linn. fruit extract. Journal of Ethnopharmacology 135: 430–3.
10. Y. BinteSadek, N. Choudhury, M. Shahriar, Biological investigations of the leaf extracts Carissa carandas, Int. J. Pharm. Res. Technol. 5 (2013) 97–105.
11. N.B.F Food, N. Board, Institute of Medicine, Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids, 2000.
12. S.V.S. Verma, H.S. Chaudhary, Effect of Carissa carandas against clinically pathogenic bacterial strains, J. Pharm. Res. 4 (10) (2012) 3769–3771, https://doi. org/10.1016/j.lwt.2018.04.012.
13. S. Rajaram, G. Ashvin, Comparative studies of phytochemical screening of Carissa carandas L, Asian J. Plant Sci. 3 (2013) 21–25. https://hal.science/hal-03696386.
14. K. Verma, D. Shrivastava, G. Kumar, Antioxidant activity and DNA damage inhibition in vitro by a methanolic extract of Carissa carandas (Apocynaceae) leaves, J. Taibah Univ. Sci. 9 (2015) 34–40, https://doi.org/10.1016/j. jtusci.2014.07.001.
15. T. Agarwal, R. Singh, A.D. Shukla, I. Waris, In vitro study of antibacterial activity of Carissa carandas Leaf extracts, Asian J. Plant Sci. Res. 2 (2012) 36–40.
16. S. Sundararajan, H. Rabe, Prevention of iron deficiency anemia in infants and toddlers, Pediatric res 89 (2021) 63–73. https://www.nature.com/articles/s41390 -020-0907-5.
17. Institute of Medicine, Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, 2001.
18. Z.A. Bhutta, J.K. Das, A. Rizvi, M.F. Gaffey, N. Walker, S. Horton, R.E. Black, Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? The lancet 382 (2013) 452–477, https://doi. org/10.1016/S0140-6736(13)60996-4.
19. International Zinc Nutrition Consultative Group (IZiNCG), Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control, 2004.
20. FAO, Save and Grow: A Policymaker's Guide to the Sustainable Intensification of Smallholder Crop Production, Rome, 2011 (also available at: http://www.fao.org/ 3/I2215E/i2215e.pdf.

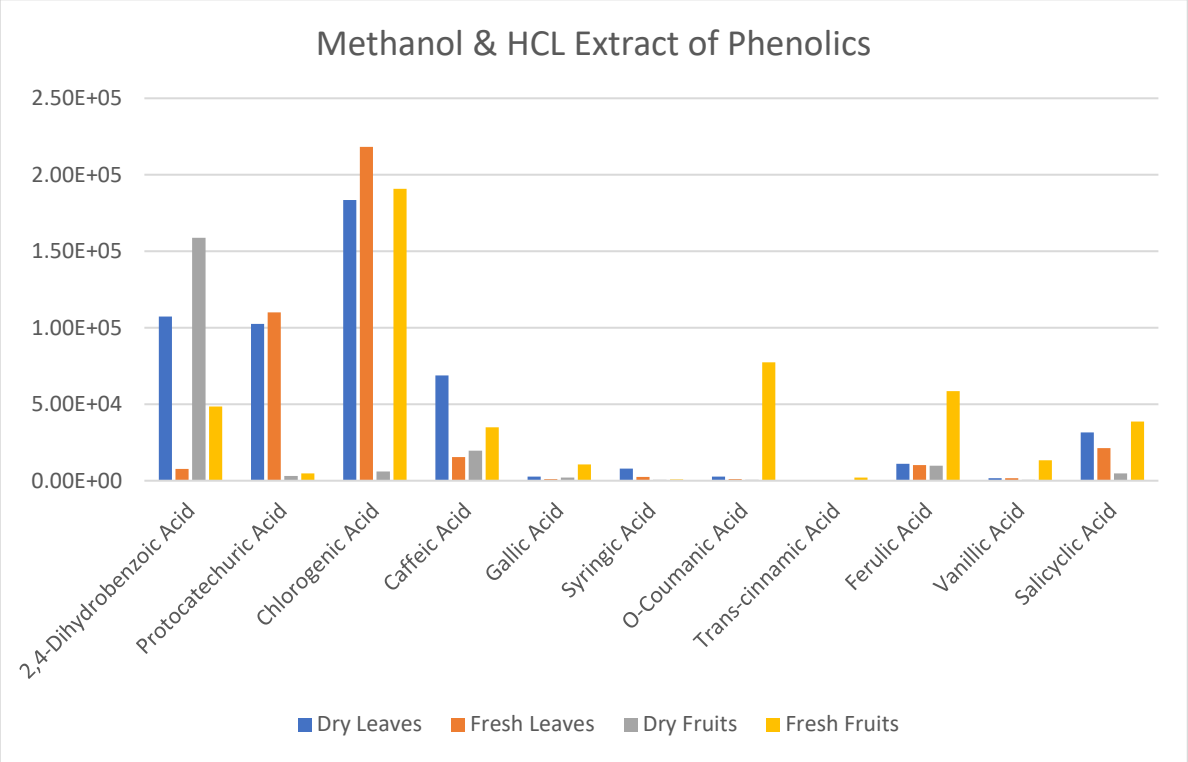
Tables and Figures



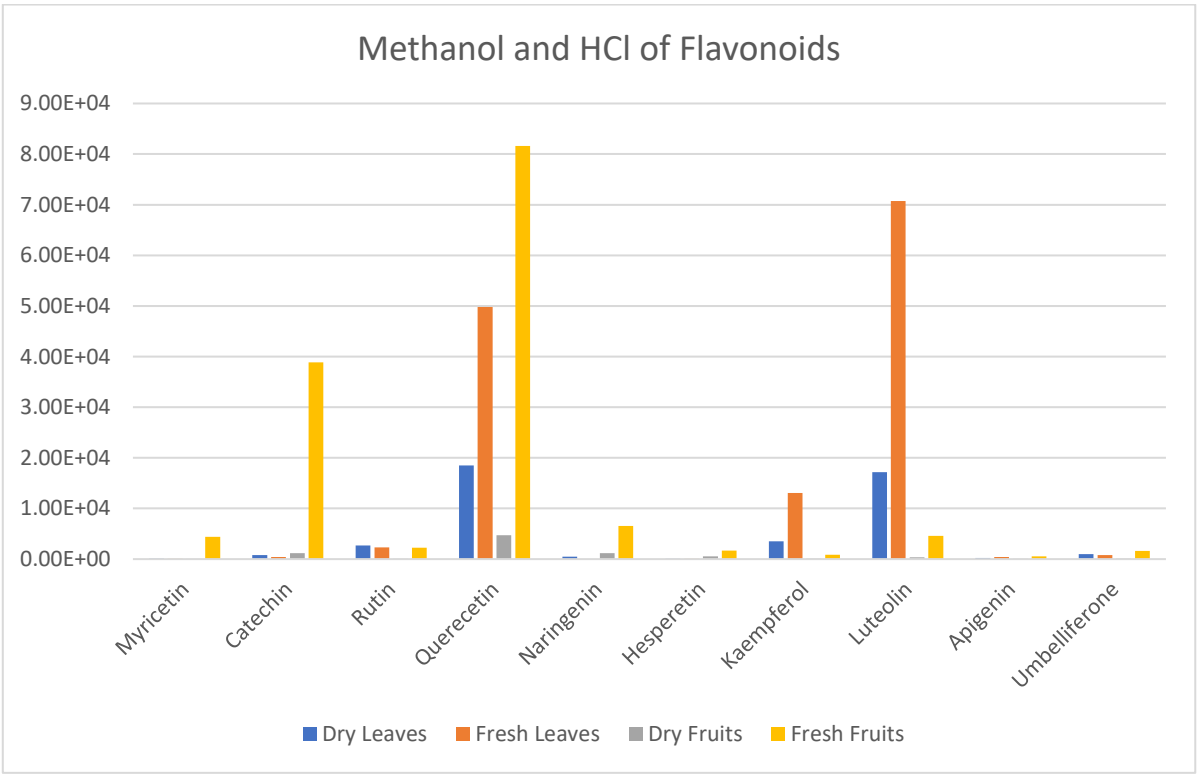
Graphic 1: Concentration of Phenolics



Graphic 2: Contraction of Flavonoids



Graphic 3: Concentration of Phenolics



Graphic 4: Concentration of Flavonoids

Legends

Table 3: Concentration of Phenolics In methanol Extraction

		Methanol Extract of Phenolics		
S. No	Name Of Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
1	2,4-Dihydrobenzoic Acid			
	Dry Leaves	2.90E+08	3.32E+05	8.73E+04
	Fresh Leaves	2.90E+06	2.97E+05	9.76E+02
	Dry Fruits	3.73E+08	3.77E+05	9.89E+04
	Fresh Fruits	4.25E+08	1.18E+05	3.60E+05
2	Protocatechuric Acid			
	Dry Leaves	1.60E+08	2.25E+05	7.11E+04
	Fresh Leaves	9.02E+05	2.97E+05	3.04E+02
	Dry Fruits	2.34E+06	3.77E+05	6.21E+02
	Fresh Fruits	3.50E+06	1.18E+05	2.97E+03
3	Chlorogenic Acid			
	Dry Leaves	1.06E+09	3.36E+05	3.15E+05
	Fresh Leaves	1.16E+09	2.97E+05	3.91E+05
	Dry Fruits	2.73E+07	3.77E+05	7.24E+03
	Fresh Fruits	9.17E+08	1.18E+05	7.77E+05
4	Caffeic Acid			
	Dry Leaves	1.89E+08	3.36E+05	5.63E+04
	Fresh Leaves	5.41E+06	2.97E+05	1.82E+03
	Dry Fruits	2.01E+07	3.77E+05	5.33E+03
	Fresh Fruits	1.35E+08	1.18E+05	1.14E+05
5	Gallic Acid			
	Dry Leaves	1.98E+06	3.32E+05	5.96E+02
	Fresh Leaves	1.08E+05	2.97E+05	3.64E+01
	Dry Fruits	4.10E+06	3.77E+05	1.09E+03
	Fresh Fruits	4.37E+07	1.18E+05	3.70E+04
6	Syringic Acid			
	Dry Leaves	5.30E+06	3.32E+05	1.60E+03
	Fresh Leaves	5.51E+05	2.97E+05	1.86E+02
	Dry Fruits	6.24E+05	3.77E+05	1.66E+02
	Fresh Fruits	3.17E+06	1.18E+05	2.69E+03

		Methanol Extract of Phenolics		
S. No	Name Of Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
7	O-Coumanic Acid			
	Dry Leaves	9.65E+07	3.32E+05	2.91E+04
	Fresh Leaves	5.06E+06	2.97E+05	1.70E+03
	Dry Fruits	4.41E+07	3.77E+05	1.17E+04
	Fresh Fruits	2.70E+08	1.18E+05	2.29E+05
8	Trans-cinnamic Acid			
	Dry Leaves	1.88E+06	3.32E+05	5.66E+02
	Fresh Leaves	1.57E+05	2.97E+05	5.29E+01
	Dry Fruits	1.25E+06	3.77E+05	3.32E+02
	Fresh Fruits	8.60E+06	1.18E+05	7.29E+03
9	Ferulic Acid			
	Dry Leaves	2.02E+07	3.32E+05	6.08E+03
	Fresh Leaves	3.90E+06	2.97E+05	1.31E+03
	Dry Fruits	1.65E+07	3.77E+05	4.38E+03
	Fresh Fruits	2.57E+08	1.18E+05	2.18E+05
10	Vanillic Acid			
	Dry Leaves	8.07E+05	3.32E+05	2.43E+02
	Fresh Leaves	1.91E+06	2.97E+05	6.43E+02
	Dry Fruits	6.61E+05	3.77E+05	1.75E+02
	Fresh Fruits	6.67E+07	1.18E+05	5.65E+04
11	Salicyclic Acid			
	Dry Leaves	8.07E+05	3.32E+05	2.43E+02
	Fresh Leaves	1.91E+06	2.97E+05	6.43E+02
	Dry Fruits	6.61E+05	3.77E+05	1.75E+02
	Fresh Fruits	6.67E+07	1.18E+05	5.65E+04

Table 4: Concentration of Flavonoids In methanol Extraction

		Methanol Extract of Flavonoids		
S.No	Name Of Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
1	Myricetin			
	Dry Leaves	5.90E+04	3.32E+05	1.78E+01
	Fresh Leaves	1.31E+05	2.97E+05	4.41E+01
	Dry Fruits	2.20E+04	3.77E+05	5.84E+00
	Fresh Fruits	1.56E+07	1.18E+05	1.32E+04

		Methanol Extract of Flavonoids		
S.No	Name Of Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
2	Catechin			
	Dry Leaves	6.22E+07	2.25E+05	2.76E+04
	Fresh Leaves	9.39E+08	2.97E+05	3.16E+05
	Dry Fruits	2.24E+06	3.77E+05	5.94E+02
	Fresh Fruits	1.69E+08	1.18E+05	1.43E+05
3	Rutin			
	Dry Leaves	9.18E+06	3.36E+05	2.73E+03
	Fresh Leaves	1.28E+07	2.97E+05	4.31E+03
	Dry Fruits	1.71E+05	3.77E+05	4.54E+01
	Fresh Fruits	8.50E+06	1.18E+05	7.20E+03
4	Querecetin			
	Dry Leaves	8.16E+06	3.36E+05	2.43E+03
	Fresh Leaves	7.47E+06	2.97E+05	2.52E+03
	Dry Fruits	2.20E+07	3.77E+05	5.84E+03
	Fresh Fruits	2.36E+08	1.18E+05	2.00E+05
5	Naringenin			
	Dry Leaves	5.20E+06	3.32E+05	1.57E+03
	Fresh Leaves	1.40E+06	2.97E+05	4.71E+02
	Dry Fruits	1.49E+07	3.77E+05	3.95E+03
	Fresh Fruits	2.09E+07	1.18E+05	1.77E+04
6	Hesperetin			
	Dry Leaves	1.87E+05	3.32E+05	5.63E+01
	Fresh Leaves	1.74E+05	2.97E+05	5.86E+01
	Dry Fruits	3.51E+06	3.77E+05	9.31E+02
	Fresh Fruits	5.24E+06	1.18E+05	4.44E+03
7	Kaempferol			
	Dry Leaves	5.40E+06	3.32E+05	1.63E+03
	Fresh Leaves	8.00E+05	2.97E+05	2.69E+02
	Dry Fruits	2.24E+06	3.77E+05	5.94E+02
	Fresh Fruits	1.10E+07	1.18E+05	9.32E+03
8	Luteolin			
	Dry Leaves	9.01E+07	3.32E+05	2.71E+04
	Fresh Leaves	3.79E+08	2.97E+05	1.28E+05
	Dry Fruits	1.58E+06	3.77E+05	4.19E+02
	Fresh Fruits	2.15E+07	1.18E+05	1.82E+04

		Methanol Extract of Flavonoids		
S.No	Name Of Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
9	Apigenin			
	Dry Leaves	1.53E+06	3.32E+05	4.61E+02
	Fresh Leaves	3.59E+05	2.97E+05	1.21E+02
	Dry Fruits	4.82E+05	3.77E+05	1.28E+02
	Fresh Fruits	1.15E+06	1.18E+05	9.75E+02
10	Umbelliferone			
	Dry Leaves	1.16E+06	3.32E+05	3.49E+02
	Fresh Leaves	1.14E+06	2.97E+05	3.84E+02
	Dry Fruits	6.05E+05	3.77E+05	1.60E+02
	Fresh Fruits	1.46E+06	1.18E+05	1.24E+03

Table 5: Concentration of Phenolics in Methanol & HCl Extraction

		Methanol & HCL Extract of Phenolics		
S.No	Name Of Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
1	2,4-Dihydrobenzoic Acid			
	Dry Leaves	5.62E+08	5.24E+05	1.07E+05
	Fresh Leaves	4.16E+07	5.36E+05	7.76E+03
	Dry Fruits	6.75E+08	4.25E+05	1.59E+05
	Fresh Fruits	2.27E+08	4.68E+05	4.85E+04
2	Protocatechuric Acid			
	Dry Leaves	5.37E+08	5.24E+05	1.02E+05
	Fresh Leaves	5.90E+08	5.36E+05	1.10E+05
	Dry Fruits	1.28E+07	4.25E+05	3.01E+03
	Fresh Fruits	2.19E+07	4.68E+05	4.68E+03
3	Chlorogenic Acid			
	Dry Leaves	9.61E+08	5.24E+05	1.83E+05
	Fresh Leaves	1.17E+09	5.36E+05	2.18E+05
	Dry Fruits	2.58E+07	4.25E+05	6.07E+03
	Fresh Fruits	8.93E+08	4.68E+05	1.91E+05
4	Caffeic Acid			
	Dry Leaves	3.61E+08	5.24E+05	6.89E+04
	Fresh Leaves	8.26E+07	5.36E+05	1.54E+04
	Dry Fruits	8.35E+07	4.25E+05	1.96E+04
	Fresh Fruits	1.63E+08	4.68E+05	3.48E+04

		Methanol & HCL Extract of Phenolics		
S.No	Name Of Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
5	Gallic Acid			
	Dry Leaves	1.41E+07	5.24E+05	2.69E+03
	Fresh Leaves	5.85E+06	5.36E+05	1.09E+03
	Dry Fruits	8.82E+06	4.25E+05	2.08E+03
	Fresh Fruits	5.02E+07	4.68E+05	1.07E+04
6	Syringic Acid			
	Dry Leaves	4.11E+07	5.24E+05	7.84E+03
	Fresh Leaves	1.33E+07	5.36E+05	2.48E+03
	Dry Fruits	2.65E+06	4.25E+05	6.24E+02
	Fresh Fruits	3.79E+06	4.68E+05	8.10E+02
7	O-Coumanic Acid			
	Dry Leaves	1.37E+07	5.24E+05	2.61E+03
	Fresh Leaves	5.99E+06	5.36E+05	1.12E+03
	Dry Fruits	2.42E+06	4.25E+05	5.69E+02
	Fresh Fruits	3.62E+08	4.68E+05	7.74E+04
8	Trans-cinnamic Acid			
	Dry Leaves	4.00E+04	5.24E+05	7.63E+00
	Fresh Leaves	1.22E+04	5.36E+05	2.28E+00
	Dry Fruits	5.25E+05	4.25E+05	1.24E+02
	Fresh Fruits	9.43E+06	4.68E+05	2.01E+03
9	Ferulic Acid			
	Dry Leaves	5.82E+07	5.24E+05	1.11E+04
	Fresh Leaves	5.46E+07	5.36E+05	1.02E+04
	Dry Fruits	4.15E+07	4.25E+05	9.76E+03
	Fresh Fruits	2.74E+08	4.68E+05	5.85E+04
10	Vanillic Acid			
	Dry Leaves	8.16E+06	5.24E+05	1.56E+03
	Fresh Leaves	8.74E+06	5.36E+05	1.63E+03
	Dry Fruits	2.22E+06	4.25E+05	5.22E+02
	Fresh Fruits	6.26E+07	4.68E+05	1.34E+04
11	Salicyclic Acid			
	Dry Leaves	1.65E+08	5.24E+05	3.15E+04
	Fresh Leaves	1.14E+08	5.36E+05	2.13E+04
	Dry Fruits	1.99E+07	4.25E+05	4.68E+03
	Fresh Fruits	1.81E+08	4.68E+05	3.87E+04

Table 6: Concentration of Flavonoids in Methanol & HCl Extraction

		Methanol and HCL Extract of Flavonoids		
S.No	NameOf Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
1	Myricetin			
	Dry Leaves	4.20E+05	5.24E+05	8.02E+01
	Fresh Leaves	2.06E+05	5.36E+05	3.84E+01
	Dry Fruits	2.85E+05	4.25E+05	6.71E+01
	Fresh Fruits	2.07E+07	4.68E+05	4.42E+03
2	Catechin			
	Dry Leaves	4.27E+06	5.24E+05	8.15E+02
	Fresh Leaves	2.13E+06	5.36E+05	3.97E+02
	Dry Fruits	4.98E+06	4.25E+05	1.17E+03
	Fresh Fruits	1.82E+08	4.68E+05	3.89E+04
3	Rutin			
	Dry Leaves	1.41E+07	5.24E+05	2.69E+03
	Fresh Leaves	1.24E+07	5.36E+05	2.31E+03
	Dry Fruits	2.31E+05	4.25E+05	5.44E+01
	Fresh Fruits	1.05E+07	4.68E+05	2.24E+03
4	Querecetin			
	Dry Leaves	9.70E+07	5.24E+05	1.85E+04
	Fresh Leaves	2.67E+08	5.36E+05	4.98E+04
	Dry Fruits	2.00E+07	4.25E+05	4.71E+03
	Fresh Fruits	3.82E+08	4.68E+05	8.16E+04
5	Naringenin			
	Dry Leaves	2.44E+06	5.24E+05	4.66E+02
	Fresh Leaves	2.13E+00	5.36E+05	3.97E-04
	Dry Fruits	4.98E+06	4.25E+05	1.17E+03
	Fresh Fruits	3.07E+07	4.68E+05	6.56E+03
6	Hesperetin			
	Dry Leaves	9.94E+04	5.24E+05	1.90E+01
	Fresh Leaves	1.02E+05	5.36E+05	1.90E+01
	Dry Fruits	2.31E+06	4.25E+05	5.44E+02
	Fresh Fruits	7.81E+06	4.68E+05	1.67E+03
7	Kaempferol			
	Dry Leaves	1.83E+07	5.24E+05	3.49E+03
	Fresh Leaves	7.01E+07	5.36E+05	1.31E+04
	Dry Fruits	3.09E+05	4.25E+05	7.27E+01
	Fresh Fruits	4.04E+06	4.68E+05	8.63E+02

		Methanol and HCL Extract of Flavonoids		
S.No	NameOf Compound	Area Of Compound	Area Of ISTD	Concentration Of Compound
8	Luteolin			
	Dry Leaves	9.01E+07	5.24E+05	1.72E+04
	Fresh Leaves	3.79E+08	5.36E+05	7.07E+04
	Dry Fruits	1.58E+06	4.25E+05	3.72E+02
	Fresh Fruits	2.15E+07	4.68E+05	4.59E+03
9	Apigenin			
	Dry Leaves	1.13E+06	5.24E+05	2.16E+02
	Fresh Leaves	2.13E+06	5.36E+05	3.97E+02
	Dry Fruits	1.48E+05	4.25E+05	3.48E+01
	Fresh Fruits	2.40E+06	4.68E+05	5.13E+02
10	Umbelliferone			
	Dry Leaves	5.21E+06	5.24E+05	9.94E+02
	Fresh Leaves	4.15E+06	5.36E+05	7.74E+02
	Dry Fruits	3.41E+05	4.25E+05	8.02E+01
	Fresh Fruits	7.59E+06	4.68E+05	1.62E+03