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Research Article

HPLC study of phenolic compounds in *Mirabilis jalapa* raw material

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Abstract

Mirabilis jalapa is a popular decorative plant valued for its beautiful multicolored flowers. Folk medicine in various countries applies *Mirabilis jalapa* as anti-microbial, anti-inflammatory, diuretic, spasmolytic drug. Chemical composition of different types of *Mirabilis jalapa* has not yet been adequately studied which is an obstacle for its application in medicine.

The qualitative composition and quantitative content of phenolic compounds were studied by the HPLC method. The performed experiment revealed presence of hydroxycinnamic acids, flavonoids, isoflavonoids and coumarins in tested herb.

The content of phenolic compounds was the highest in *Mirabilis jalapa* flowers, counting as much as 2977.41 ± 59.55 μg/mg. Total content of phenolic compounds in *Mirabilis jalapa* herb was 304.25 ± 6.08 μg/mg, in fruits – 67.92 ± 1.36 μg/mg, and in roots – 12.44 ± 0.25 μg/mg.

Quantitatively neochlorogenic acid dominated in flowers, chlorogenic acid in fruits, whereas *Mirabilis jalapa* herb mostly contained rutin and hyperoside.

The obtained results will be useful in the development of quality control methods for *Mirabilis jalapa* herb and manufacture of drug preparations on its basis.

Keywords

Mirabilis jalapa, four-o'clocks, phenolic compounds, HPLC

Introduction

Mirabilis jalapa Linn. (clavillia or four-o'clocks) belongs to *Nyctaginaceae* family. This decorative plant is cultivated all over the world. *Mirabilis jalapa* flowers bear pink, yellow, red, orange, white colors or several colors simultaneously (Akanji et al. 2016).

Mirabilis jalapa is also known for its healing properties. Folk medicine in various countries applies it to treat dysentery, diarrhea, inflammations, muscular pain, diabetes, urogenital disorders (Ramesh and Mahalakshmi 2014).

Mirabilis jalapa herb, leaves, flowers, fruits and roots contain phenolic compounds, steroids and triterpenoids, carbohydrates, lipids, amino acids (Lai et al. 2008; Yakubu et al. 2019; Zachariah et al. 2012).

Mirabilis jalapa herb and roots contain phytosterols: β-sitosterol, stigmasterol, brassicasterol, ursolic, oleanolic and betulinic acids (Siddiqui et al. 1994; Gogoi et al. 2016). *Mirabilis jalapa* roots and leaves contain trigonelline which demonstrates anti-diabetic activity (Sathe and Dighe 2017; Subramanian et al. 2014). Rotenoids from *Mirabilis jalapa* roots show cytotoxic activity (Xu et al. 2010).

Pharmacological researchers in various countries confirmed anti-bacterial, anti-fungal, anthelmintic, anti-inflammatory, diuretic activity (Gogoi et al. 2016; Hajji et al. 2010; Salman et al. 2015; Singh et al. 2010; Yakubu et al. 2019).

Nigerian scientists established anti-malaria activity of *Mirabilis jalapa* leaves extract (Akanji et al. 2016), whereas scientists from Mexico found spasmolytic effect of *Mirabilis jalapa* flowers extract (Aoki et al. 2008).

The object of the present work was the study of phenolic compounds in *Mirabilis jalapa* herb, flowers, fruits and roots.

Materials and methods

Sample preparation

Plant. The powdered materials of plant (0.3 g) were weighed into a volumetric flask and extracted with methanol (10 mL) in an ultrasonic bath at room temperature (20 ± 2 °C) for 20 min. The solutions were filtered through a membrane filter (0.45 μm) prior to use.

HPLC conditions. Chromatographic separation of phenolic compounds was carried out using an ACE C18 column (250 mm × 4.6 mm, 5.0 μm; Pennsylvania, USA). Elution was performed at a flow rate of 1 ml/min. The mobile phase consisted of 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B). A linear gradient program was applied as follows: 0–8 min, 5–15% B; 8–30 min, 15–20% B; 30–48 min, 20–40% B; 48–58 min, 40–50% B; 58–65 min, 50%; 65–66 min, 50–95% B. The column temperature was constant 25 °C. The injection volume of the sample solution was 10 μL. The chromatograms were recorded at different wavelengths according to substances. Phenolic acids were quantified at 320 nm, flavonoids and ononin at 350 nm, coumarin at 275 nm.

Instrument. Liquid chromatography separation was performed using a Shimadzu Nexera X2 LC-30AD HPLC system (Shimadzu, Japan) composed of a quaternary pump, an on-line degasser, a column temperature controller, the SIL-30AC autosampler (Shimadzu, Japan); the CTO-20AC thermostat (Shimadzu, Japan) as well as the SPD-M20A diode array detector (DAD). Other instruments such as Ultrasonic Cleaner Set for ultra-sonication using (Wise Clean WUC-A06H, Witeg Labortechnik GmbH, Germany), Libra UniBloc AUW120D (Shimadzu Analytical Scale, Japan); class A analytical vassals that meets evaluation 2019).

The identification of the chromatographic peaks was achieved by comparing the retention times and spectral characteristics (λ = 200–600 nm)of the eluting peaks with those of the reference compounds (Table 1). The compounds identified were confirmed by spiking the sample with the standard compound and monitoring the changes in the peak shape and spectral characteristics. For quantitative analysis, a calibration curve was obtained by the injection of known concentrations of different standard compounds. The concentrations of phenolic compounds identified in the extracts were within the limits of the calibration curves.

The following validation characteristics of method were evaluated as specificity, reproducibility of results (accuracy), limits of detection and quantitative evaluation, linearity (Tables 2, 3).

The method specificity was confirmed by coincidence between peak retention time of identified phenolic compounds and the corresponding peak retention time in standard samples and divisibility of substance peaks in chromatogram.

The limit of detection (LOD) may be expressed as: DL = 3.3 *σ/S, where σ = the standard deviation of the response, S = the slope of the calibration curve. The limit of quantification (LOQ) may be expressed as DL = 3.3 *σ/S, where σ = the standard deviation of the response, S = the slope of the calibration curve. The determined LOQ was within the range from 14.00 ng/mL to 420.00 ng/mL. The obtained results confirmed method eligibility for quantitative evaluation of phenolic compounds.

The correlation coefficient (R^2) was always above 0,999, which confirmed the linearity of quantitative evaluation method.

The total content of phenolic compounds was calculated by summing the identified components.

Results and discussion

The results of experiment enabled the identification of hydroxycinnamic acids, flavonoids, isoflavonoids and coumarins in tested *Mirabilis jalapa* herb.

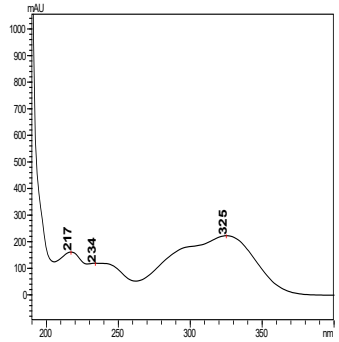
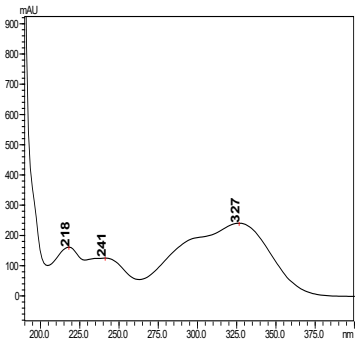
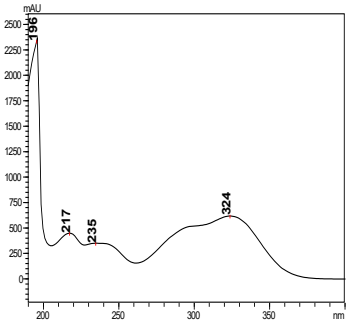
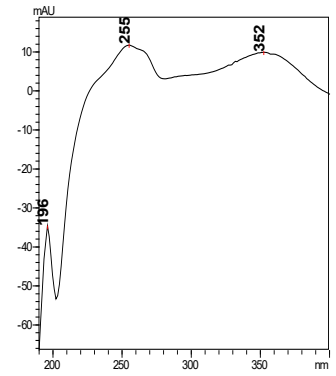
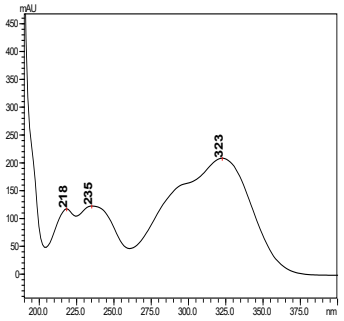
The HPLC chromatogram of *Mirabilis jalapa* flowers is specified in Fig. 1, of roots in Fig. 2, of fruits in Fig. 3 and herb in Fig. 4.

Nine phenolic compounds were detected in *Mirabilis jalapa* flowers, namely: hydroxycinnamic acids (neochlorogenic, caffeic, ferulic acids), flavonoids (rutin, hyperoside, isoquercirtrin, avicularin), isoflavonoids (ononin) and coumarins (coumarin). Eight compounds were identified in *Mirabilis jalapa* herb: hydroxycinnamic acids (neochlorogenic, chlorogenic, caffeic, ferulic acids), flavonoids (rutin, hyperoside, isoquercirtrin) and coumarins (coumarin). Among four phenolic compounds found in *Mirabilis jalapa* fruits were two hydroxycinnamic acids (chlorogenic and caffeic acids), one flavonoid (hyperoside) andone isoflavonoid (ononin). In roots of this plant only hydroxycinnamic acids (caffeic and ferulic acids) were detected.

The quantitative content of phenolic compounds as determined by the HPLC method in *Mirabilis jalapa* raw material is presented in Table 4.

The highest content of phenolic compounds was found in *Mirabilis jalapa* flowers, as much as 2977.41 μg/mg. Among hydroxycinnamic acids the most abundant was neochlorogenic acid (2060.10 μg/mg). The amount of flavonoids in flowers was 749.80 μg/mg, mostly avicularin (322.30 μg/mg), hyperoside (292.67 μg/mg) and rutin (131.63 μg/mg). Coumarin and ononin content in

Table 1. Retention time, detected at wavelength and UV spectrum of identified phenolic compounds.

Compound	Retention time, min	Detected at wavelength λ, nm	UV spectrum
Neochlorogenic acid	9.86 ± 0.20	320	
Chlorogenic acid	11.96 ±0.30	320	
Caffeic acid	14.18 ± 0.30	320	
Rutin	21.48 ± 0.50	353	
Ferulic acid	23.16 ± 0.50	320	

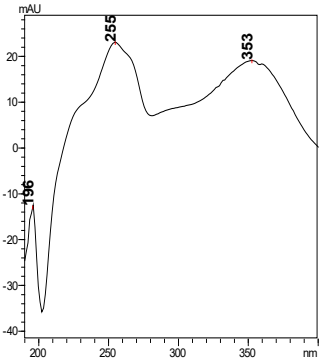
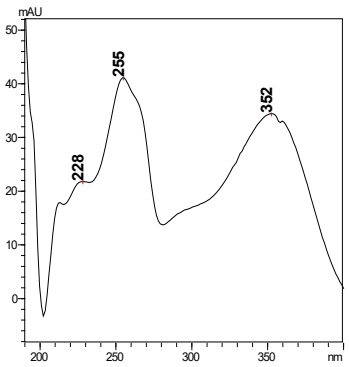
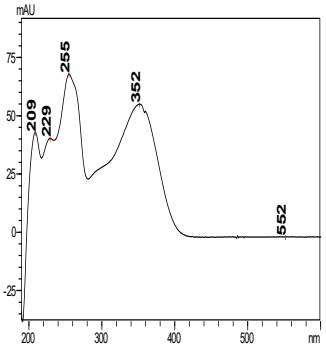
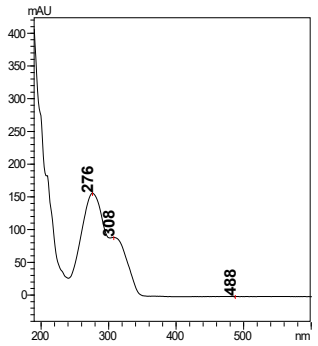
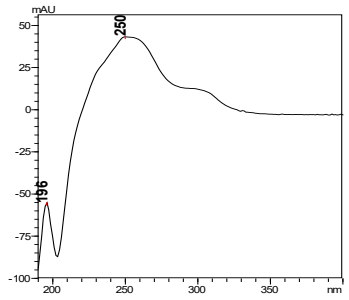
Compound	Retention time, min	Detected at wavelength λ, nm	UV spectrum
Hyperoside	23.89 ± 0.30	350	
Isoquercitrin	24.83 ± 0.20	350	
Avicularin	30.94 ± 0.65	350	
Coumarin	33.77 ± 0.30	275	
Ononin	39,52 ± 0.20	350	

Table 2. Linearity, LOD, LOQ of the HPLC method for the quantitative evaluation of phenolic compounds.

Compound	Calibration curve	Correlation coefficient R^2 (n = 6)	Linear range (µg/mL)	RSD, %	LOD[b] (µg/mL)	LOQ[c] (µg/mL)
Neochlorogenic acid	f(x) = 30834.6*x-613.100	0.9999	0.38–48.00	1.18	0.03	0.08
Chlorogenic acid	f(x) = 29930.2*x-538.361	0.9999	0.36–46.00	1.29	0.02	0.07
Caffeic acid	f(x) = 57646.8*x-3853.48	0.9999	0.72–91.92	1.56	0.02	0.06
Ferulic acid	f(x) = 54955.4*x-638.345	0.9999	0.44–56.50	1.60	0.03	0.08
Rutin	f(x) = 16072.5*x+1499.73	0.9999	0.16–20.24	1.07	0.09	0.29
Hyperoside	f(x) = 22498.4*x+2508.57	0.9999	0.21–27.04	1.19	0.07	0.23
Isoquercitrin	f(x) = 24139.7*x+3904.44	0.9999	0.35–44.56	1.02	0.07	0.22
Ononin	f(x) = 37456.2*x+5132.42	0.9998	0.21–26.24	1.29	0.05	0.15
Avicularin	f(x) = 16508.5*x-158.335	0.9999	0.16–10.39	1.35	0.004	0.01
Coumarin	f(x) = 34481.8*x+42631.1	0.9999	1.85–237.50	0.81	0.14	0.42

Table 3. Precision of the HPLC method for the quantitative evaluation of phenolic compounds.

Compound	Concentrate (µg/mL)	Deviation (%)	Intra-Day (n = 3)		Inter-Day (n=3)	
			RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
Neochlorogenic acid	6.05	100.17	0.87	100.35	0.88	101.16
	24.12	98.58	1.25	99.17	0.97	102.37
	48.43	100.14	1.22	100.25	0.71	101.27
Chlorogenic acid	5.75	100.69	1.31	101.12	0.38	98.40
	23.20	99.58	0.42	99.08	0.73	99.43
	46.58	101.91	0.96	100.27	0.48	98.24
Caffeic acid	11.49	100.01	1.05	102.02	0.52	98.49
	45.96	99.39	1.08	98.78	0.67	99.73
	91.92	100.17	0.64	100.35	0.95	98.17
Ferulic acid	7.06	99.11	0.68	100.22	0.90	98.29
	28.25	99.60	0.93	98.20	0.29	99.31
	56.50	100.12	1.22	100.24	0.46	98.28
Rutin	2.53	100.18	1.26	100.35	0.62	100.15
	10.12	100.65	1.29	101.12	0.80	99.21
	20.24	99.78	0.76	99.56	1.14	100.94
Hiperoside	3.433	100.78	0.99	101.06	0.86	98.95
	13.72	100.72	0.50	101.04	0.70	99.07
	26.92	99.77	0.42	99.53	0.80	100.97
Isoquercitrin	5.57	100.13	0.86	100.26	0.41	100.23
	22.28	99.64	1.12	101.27	0.98	99.24
	44.56	97.79	0.80	99.58	0.91	100.92
Ononin	3.28	100.56	0.81	100.12	0.32	98.39
	13.12	100.72	0.69	100.44	0.75	99.08
	26.24	99.75	0.84	99.50	0.82	100.00
Avicularin	0.32	100.15	1.02	98.75	1.06	100.63
	1.29	99.59	0.78	101.78	0.97	99.12
	5.19	99.41	0.94	100.10	0.71	99.95
Coumarin	1.85	99.82	0.79	100.12	0.98	100.02
	7.42	99.57	0.86	99.34	1.07	99.87
	29.68	100.11	0.90	98.99	1.10	99.32

Table 4. The content of phenolic compounds in *Mirabilis jalapa* raw material.

Compound	Flowers		Roots		Fruits		Herb	
	Retention time, min	Quantitative content, µg/mg	Retention time, min	Quantitative content, µg/mg	Retention time, min	Quantitative content, µg/mg	Retention time, min	Quantitative content, µg/mg
Neochlorogenic acid	9.92	2060.10 ± 41.20	–	–	–	–	9.94	69.87 ± 3.49
Chlorogenic acid	–	–	–	–	10.98	51.73 ± 1.03	11.36	5.30 ± 0.11
Caffeic acid	14.04	76.77 ± 1.54	13.88	4.40 ± 0.09	14.26	10.13 ± 0.20	14.05	8.60 ± 0.17
Rutin	21.09	131.63 ± 2.63	–	–	–	–	21.23	110.33 ± 2.21
Ferulic acid	22.73	79.37 ± 1.59	23.67	4.57 ± 0.09	–	–	22.80	12.93 ± 0.26
Hyperoside	23.66	292.67 ± 5.85	–	–	23.67	4.37 ± 0.09	23.77	93.20 ± 1.86
Isoquercitrine	24.89	3.20 ± 0.06	–	–	–	–	24.87	1.00 ± 0.02
Avicularin	31.55	322.30 ± 6.45	–	–	–	–	–	–
Coumarin	33.55	8.96 ± 0.18	–	–	–	–	33.62	3.02 ± 0.06
Ononin	39.58	2.41 ± 0.05	–	–	39.41	1.69 ± 0.03	–	–

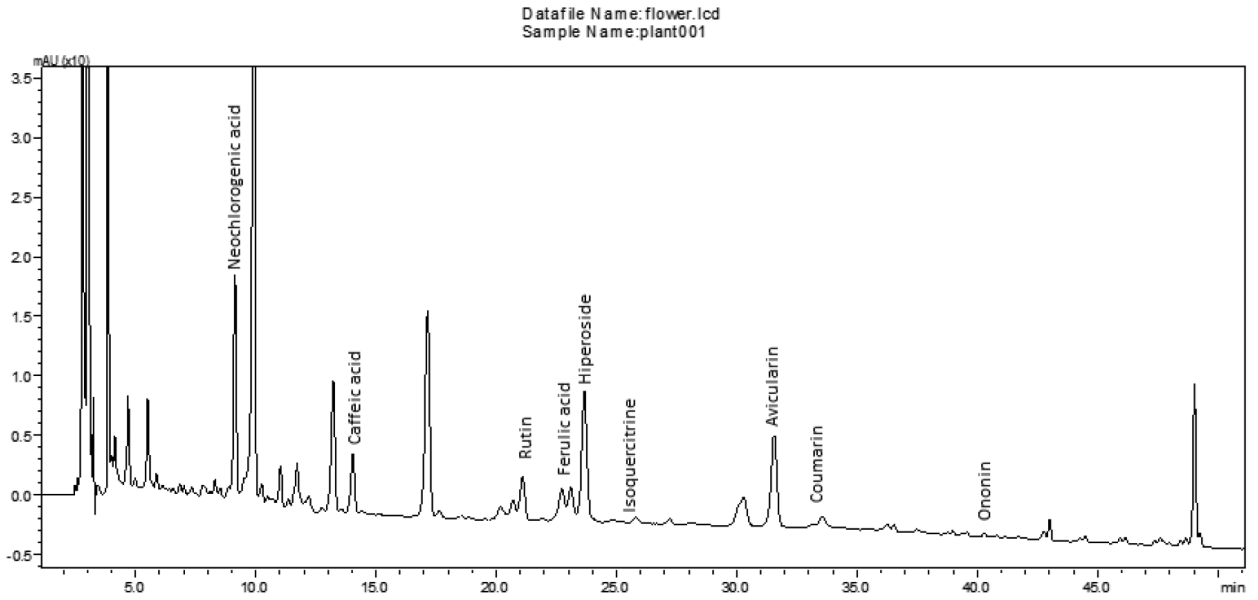


Figure 1. The HPLC chromatogram of phenolic compounds in *Mirabilis jalapa* flowers.

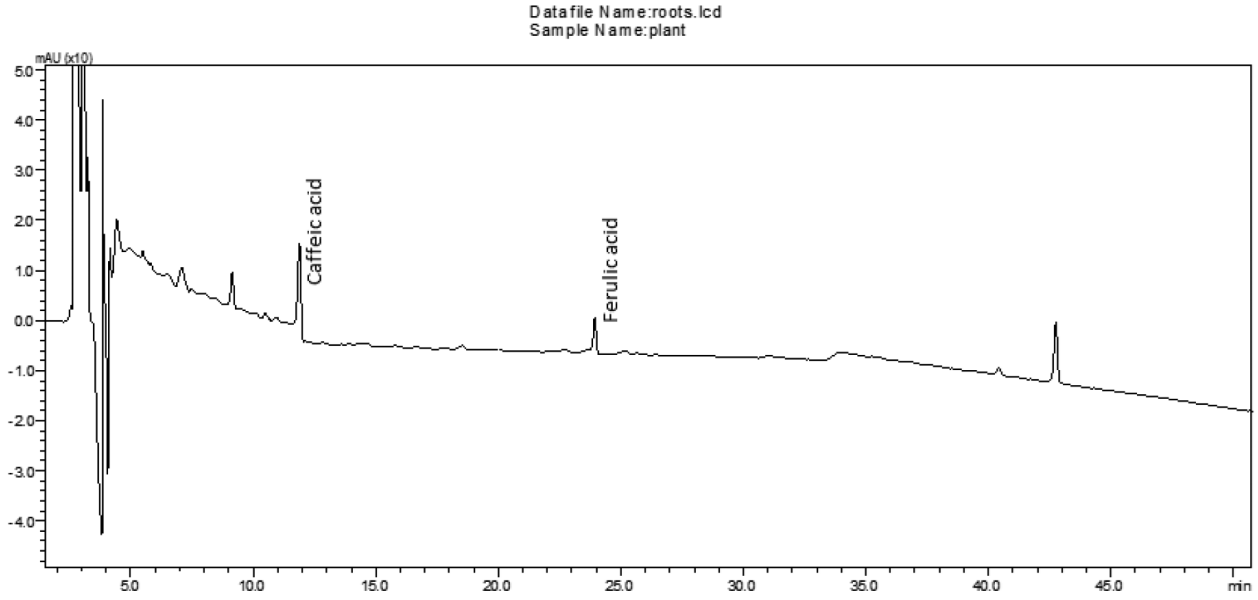


Figure 2. The HPLC chromatogram of phenolic compounds in *Mirabilis jalapa* roots.

Mirabilis jalapa flowers was negligible – 8.96 μg/mg and 2.41 μg/mg respectively.

Mirabilis jalapa herb contained 304.25 μg/mg phenolic compounds, including 204.53 μg/mg flavonoids, 96.70 μg/mg hydroxycinnamic acids and 3.02 μg/mg coumarins. The most abundant in the herb were rutin (110.33 μg/mg) and neochlorogenic acid (69.87 μg/mg).

In *Mirabilis jalapa* fruits chlorogenic acid dominated (51.73 μg/mg). The total content of identified phenolic compounds in fruits was 67.92 μg/mg.

The lowest content of phenolic compounds among tested parts of herb was in roots – 12.44 μg/mg. The amounts of ferulic and caffeic acids in these was almost identical (4.57 μg/mg and 4.40 μg/mg respectively).

Conclusion

Our experiment enabled the identification of flavonoids, isoflavonoids, hydroxycinnamic acids and coumarins in *Mirabilis jalapa* raw material. The neochlorogenic acid (2060.10 μg/mg) dominated in *Mirabilis jalapa* flowers. The rutin (110.33 μg/mg) and hyperoside (93.20 μg/mg) prevailed in herb. The chlorogenic acid content in *Mirabilis jalapa* fruits was 51.73 μg/mg, much more than all other identified phenolic compounds in this part of herb. The least of all phenolic compounds were found in roots.

The obtained results will be useful in the development of quality control methods for *Mirabilis jalapa* herb and manufacture of drug preparations on its basis.

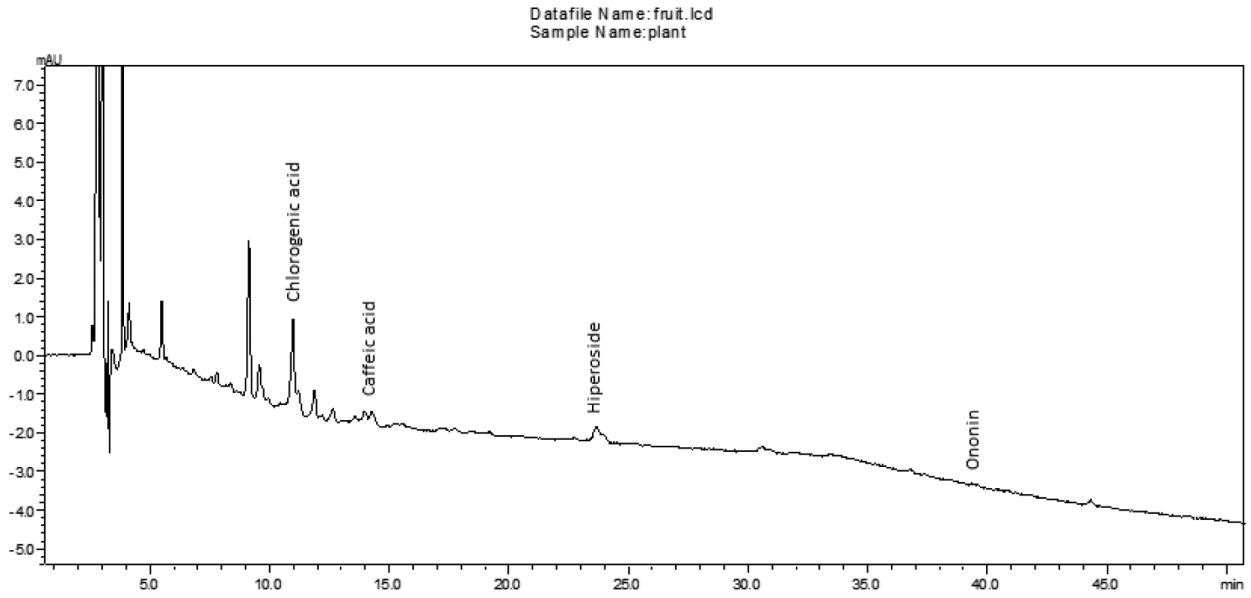


Figure 3. The HPLC chromatogram of phenolic compounds in *Mirabilis jalapa* fruits.

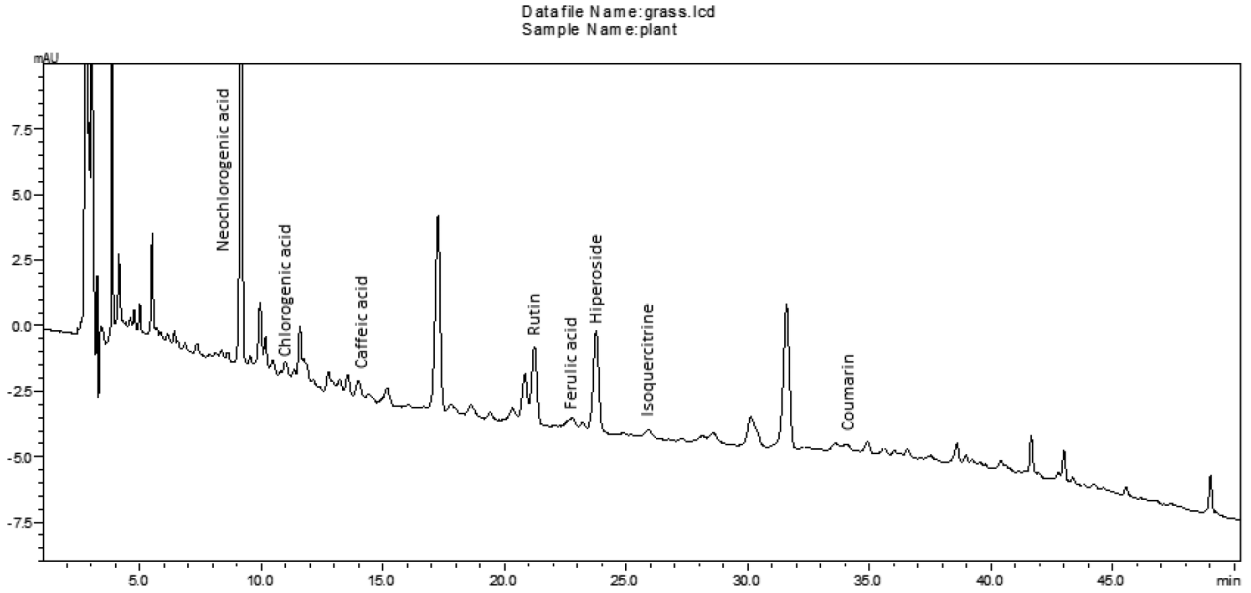


Figure 4. The HPLC chromatogram of phenolic compounds in *Mirabilis jalapa* herb.

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