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PHYTOCHEMICAL ANALYSIS, DNA BARCODING AND DNA PROTECTIVE ACTIVITY OF *FERULAGO CASSIA* BOISS.

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Abstract

Chemical composition of the methanol extracts of *Ferulago cassia* Boiss. were identified by HPLC PDA and their DNA protective activity pattern determined. Regarding the chemotaxonomic evaluation, genetic analysis (barcoding) of species was carried out to contribute to differentiation of *Ferulago* species or phylogenetically close to other taxa. Chicoric acid and chlorogenic acid were found to be major compounds in both flowers and leaves extracts of *F. cassia.* Both methanol extracts were tested for DNA protective activity. Both of the extracts completely protected the pBluescript II SK (+) plasmid DNA from oxidative stress induced by UV and H_2O_2 in a DNA damage inhibition assay in which flower extract showed slightly more protection. The extract showed a great potential for DNA protection. ITS (internal transcribed spacer) sequence of *F. cassia* was obtained for the first time for DNA barcoding. The phylogenetic tree showed that *F. cassia* is closely related to *F. macrocarpa*.

Key words: *Ferulago cassia,* Phenolic compounds, DNA barcoding, Phylogenetic Tree, DNA protective activity.

Introduction

The genus *Ferulago* W. D. J. Koch, a member of the family Apiaceae, is represented by about 50 species worldwide. Distribution of this genus in the world includes regions such as Europe (except northern part), central and South-western Asia, Caucasia, and Northern and North-western Africa (Akalın & Kızılarslan, 2013). It was recorded in the 4 volumes of Flora of Turkey that there were 28 species of the genus *Ferulago*, of which 14 were endemic (Peşmen, 1972). Over the last 20 years, several studies conducted on *Ferulago* genus in Turkey prove that the speciation of this genus continues and the number of species proceed to increase due to different ecosystems, habitats, and substrate types (Gürdal *et al*., 2021). Recently, this number has increased to 35, 19 of which are endemic, with the discovery of the new endemic *Ferulago* species namely *F. akpulutii* in 2021 (Peşmen, 1972; Gürdal *et al*., 2021; Saya, 2012). The fact that Turkey is home to many *Ferulago* species, most of which are endemic, supports the idea that Turkey is the main centre of diversity of the genus (Akalın & Özhatay, 1996).

In Turkish folk medicine, various parts of *Ferulago* species have been used for many diseases. Roots of some members of the genus *Ferulago* have been employed as aphrodisiac and for curing cancers and dermatological illness. Aerial parts of some species have been reported to be utilized as digestive, tonic, sedative, flavor, vermicidal, immunostimulant, and anti-bronchitis (Karakaya *et al*., 2019). *Ferulago cassia* known as "şeytan kişnişi" is a perennial flowering plant. It grows wild in Adana, Kahramanmaraş, Mersin and Hatay, Southern Anatolia at Turkey (Peşmen, 1972; Saya, 2012). The people living in some regions have utilized their seeds to treat eye pains and increase the milk secretion (Demirci & Ozhatay, 2012).

In the studies carried out to date, roots and aerial parts of *Ferulago* taxa, especially some species such as *F. angulata*, *F. campestris*, and *F. capillaris* (Saleh *et al*., 2021), have been the subject of extensive phytochemical studies (Badalamenti *et al*., 2021). Many substances have been identified such as coumarins, sesquiterpenes aryl esters, daucane derivatives, phenol derivatives, and flavonoids in the *Ferulago* species. In addition, essential oil constituents of several *Ferulago* species have been determined (Baser *et al*., 2002). Coumarins are the most prevalent secondary metabolite constituents in the *Ferulago* genus (Rahimpoura *et al*., 2021; Saleh *et al*., 2021). Conversely, the flavonoid compounds found in plants are too limited and have usually been identified in the aerial parts of the genus (Süzgeç-Selçuk & Dikpınar, 2021). Coumarin compounds have been isolated from *F. cassia* (Karakaya *et al*., 2019) and its essential oil has been analyzed (Sanli *et al*., 2020). Moreover, *F. cassia* was found to be significant anticancer (Bakar *et al*., 2021), antioxidant and anticholinesterase activities (Karakaya *et al*., 2019).

Peucedanum L., *Ferula* L. and *Ferulago* W. D. J. Koch are three genus that are phylogenetically close to each other. Due to the similarity of their external appearance, all of them are used for the same purposes among the people. The similarity between the three genera is very high, it is as difficult to distinguish the genera from each other as it is to distinguish the species (Akalın & Özhatay, 1996). DNA barcoding has been used as a useful tool in floristic studies especially for the identification of the specimens in case of lack of diagnostic characters (Aghayeva *et al*., 2021). Especially, the usage of ribulose-1,5-bisphosphate carboxylase/ oxygenase (rbcL), Maturase K (matK), trnH-psbA, intergenic spacer region and nuclear ribosomal DNA; plant DNA barcode markers to solve basic questions, the most common over the last decade (Kress, 2017). The effectiveness of internal transcribed spacer (ITS) region containing ITS1, 5.8 and ITS2 genes were previously studied and the results showed superior results in intra- and interspecific divergence and DNA barcoding gap assessments in Apiaceae (Liu *et al*., 2014), so in this

study, we amplified and sequenced the ITS region of *F. cassia* for DNA barcoding. This was the first sequence data for *F. cassia* and this result will help further investigators for identifying the plant sample and trying to understand the phylogenetic relationship of the genus *Ferulago*.

The available information indicates that leaves and flowers of *F. cassia* have not been evaluated comprehensively for phenolic compounds before. In addition, its DNA protective activity has not been studied before. In this study, apart from these parameters, it is aimed to provide further benefit in the differentiation of species by giving DNA sequences.

Material and Methods

Plant materials: The aerial parts of *F. cassia* Boiss. were collected from the Adıyaman, Turkey (37°49'14.60" N, 38°03'10.91" E), at their flowering stage, on 18 May 2021. Plant identity was verified by Dr. Ömer Kılıç and voucher specimens were deposited in the Herbarium of Hacettepe University Faculty of Pharmacy, Turkey (Voucher Number: HUEF21046).

Extraction procedure: The specimens were dried at room temperature and divided into two parts; flowers and leaves. 5 g milled from the each part of the plant materials were subjected to an extraction process through 100 mL methanol maceration (24 h at room temperature). The maceration process was repeated twice, and the combined extracts were filtered through filter paper, and they were then evaporated to dryness under vacuum at 40°C with a rotary evaporator. Percentage of extract yield in flowers extract was found as 33.31% and 20.39%, as obtained from leaves extract.

HPLC analysis of phenolic compounds

Chemicals and reagents: Chemical standards of gallic acid (CAS number 149-91-7; purity≥98%), 4-hydroxybenzoic acid (CAS number 99-96-7; purity≥99%), chlorogenic acid (CAS number 327-97-9; purity≥95%), vanilic acid (CAS number 121-34-6; purity≥97%), caffeic acid (CAS number 331-39-5; purity≥98 %), epicatechin (CAS number 490-46-0; purity≥98%), p-coumaric acid (CAS number 501-98-4; purity≥98), salicylic acid (CAS number 69-72-7; purity≥99%), rutin (CAS number 207671-50-9; purity≥94%), chicoric acid (CAS number 6537-80-0; purity≥95%), cinnamic acid (CAS number 140-10-3; purity≥99%), quercetin (CAS number 117-39-5; purity≥95%), and naringenin (CAS number 67604-48-2; purity≥95%) were purchased from Sigma-Aldrich (St Louis, MO, USA). Ferulic acid (CAS number 1135-24-6; purity≥99 %), and apigenin-7-*O*-glucoside (CAS number 578-74-5; purity≥97%) were obtained from Supelco (Darmstadt, Germany). All other solvents and reagents used were of HPLC and analytical grade.

HPLC-PDA Conditions and determination of phenolic acids: HPLC analysis of phenolic compounds were performed on a Shimadzu Nexera-i LC-2040C 3D Plus system which was equipped with a photodiode-array detector (PDA) at 254 nm wavelength. The separation was executed on Phenylhexyl column (4.6 x 150 mm, 3 µm (UP), (GL Sciences InterSustain Made in Japan). The method used in the analysis was developed and validated by Ataseven *et al*., (Ataseven *et al*., 2021). The linearity, limit of detection (LOD), and limit of quantification (LOQ) values of the adopted method were evaluated and then used for the analysis. The analytical parameters of the method are represented in Table 1, together with retention times. The injection volume was set to 10 µL. The analysis of the phenolic compounds were carried out at 30°C using 2 linear gradients of 0.1% formic acid (Solvent A) and acetonitrile (Solvent B) The composition of the gradient was (A:B), 95:5 at 0 min, 90.5:9.5 at 7 min, 83:17 at 20 min, 40:60 at 35 min, 100:0 at 40 min with flow rate of 1 mL/min. Identification and quantitative analysis of 15 phenolic compounds were done by comparison with standards.

Table 1. Retention time (RT), calibration curves, regression coefficient (R2), limits of detection (LOD) and quantification (LOQ) for the standard phenolic compounds.

No.	Compounds	RT (min)	Calibration equation	R^2	LOD (mg/L)	LOQ (mg/L)
1.	Galllic acid	4.352	y=29799.9x+6494.60	0.9995	0.7440	2.2547
2.	4-Hydroxybenzoic acid	10.217	y=40036.7x+1238.33	0.9995	0.6445	1.9531
3.	Chlorogenic acid	12.073	y=28066.0x+25870.2	0.9990	1.3687	4.1476
4.	Vanillic acid	12.437	y=48654.4x-26981.1	0.9997	1.0565	3.2017
5.	Caffeic acid	12.850	y=16914.9x-1409.46	0.9972	3.1730	9.6153
6.	Epicatechin	14.150	y=4788.08x+789.457	0.9986	8.4615	25.6410
7.	p-Coumaric acid	18.486	y=64013.4x-19190.2	0.9933	1.3807	4.1841
8.	Ferulic acid	20.971	y=46665.0x-14606.2	0.9928	1.3865	4.2016
9.	Salicylic acid	21.929	y=23472.2x+25113.1	0.9994	4.6906	14.2140
10.	Rutin	23.494	y=17392.1x-5957.13	0.9959	2.1258	6.4420
11.	Chicoric acid	27.011	y=7443.82x+72484.6	0.9887	3.9639	12.0120
12.	Apigenin 7-*O*-glucoside	27.574	y=39321.9x+1685.18	0.9993	0.7295	2.2107
13.	Cinnamic acid	30.234	y=75026.0x-11276.0	0.9988	0.6776	2.0533
14.	Quercetin	32.008	y=26403.4x+1558.71	0.9997	1.2724	3.8560
15.	Naringenin	34.939	y=24207.0x+2212.93	0.9920	1.3147	3.9840

Table 2. PCR conditions of the amplification of *F. cassia* ITS region.

Step	Temperature	Time	Cycle
Initial denaturation	94ºC	5 min	1 cycle
Denaturation	94ºC	1 min	
Annealing	50ºC	1 min	35 cycle
Elongiation	72ºC	1 min	
Final elongiation	72ºC	5 min	1 cycle

Table 3. The quantity of determined phenolic compounds in methanol extracts from flowers and leaves of *F. cassia* analysed by HPLC-PDA (mg/g extract).

No	Compounds	Flowers	Leaves
1.	Galllic acid	-	0.088
2.	4-Hydroxybenzoic acid	-	0.166
3.	Chlorogenic acid	41.692	99.246
4.	Vanillic acid	-	-
5.	Caffeic acid	-	1.706
6.	Epicatechin	-	0.151
7.	p-Coumaric acid	0.316	0.314
8.	Ferulic acid	-	-
9.	Salicylic acid	-	-
10.	Rutin	6.003	4.158
11.	Chicoric acid	159.754	107.754
12.	Apigenin 7-*O*-glucoside	6.747	1.432
13.	Cinnamic acid	0.705	-
14.	Quercetin	-	-
15.	Naringenin	-	-

DNA Isolation and PCR: DNA isolation was performed directly from dried samples by using DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Universal ITS4 (White *et al*., 1990) and ITS 5m (Sang *et al*., 1995) primers were used for DNA barcoding of ITS (internal transcript spacer) region. PCR was performed in 200 µL a sterile nuclease-free microcentrifuge tube with a reaction volume of 50 µL containing; 10X Taq Buffer with $(NH_4)_2SO_4$, 10 µM of each primer, gDNA (˜20 ng), 200 µM dNTP mix (Thermo Fisher Scientific.), %3 filter sterilized dimethyl sulfoxide (DMSO), Taq DNA polymerase (Thermo Fisher Scientific.), $MgCl_2$ (1.5 mM). PCR conditions were shown in Table 2. A sample without DNA was also used as negative control. 5 µL of the PCR products were mixed with 6X loading dye containing bromophenol blue and loaded on a 0.8% agarose gel supplemented with 0.5 µg/mL ethidium bromide. Electrophoresis was run at 90 V for 45 min and visualized via Quantum ST5 Gel Documentation system. The PCR product was sequenced commercially by a biotechnology company SENTEGEN (Ankara).

DNA Barcoding and phylogenetic tree: Bioedit bioinformatic tool was used to analyse the sequences especially for obtaining contigs of the forward and reverse sequences, aligning the sequences for phylogenetic tree (Hall, 1999). The blast analysis was run by using the NCBI database in order to validate the sequence belonged to *F. cassia* (Anon., 1988). Neighbor joining (NJ) tree was conducted in addition to the confirmation of the nuclear ribosomal DNA (nrDNA) ITS sequence of *F. cassia*, to reveal its position along with a selection of sequences retrieved from GenBank which were belonged to the genus *Ferulago* and closely related taxa by using PAUP 4.0 (Swofford, 2003). A total set of 39 species, including *Heracleum platytaenium* (Accession no: DQ468078.1) as outgroup and some related taxa including *Eriocycla, Azilia, Prangos, Pseudopimpinella, Diplotaenia, Bilacunaria, Lomatium, Ligusticum, Ferula, Peucedanum* and some *Ferulago* species were analyzed to evaluate clade support, bootstrap (Felsenstein, 1985) analyses were performed in PAUP (Swofford, 2003) with 1000 replicates. The Genbank accession numbers of the reference sequences were mentioned in the NJ tree figure (Fig. 4).

DNA Protective activity assay: DNA protective activity assay was performed by using pBluescript II SK(+) (Stratagene) plasmid. Plasmid isolation was performed from the overnight culture of *Escherichia coli* Dh5α cells carrying pBluescript II SK(+) (Stratagene) plasmid. Freshly prepared Luria-Bertani (LB) medium supplemented with 100 µg/mL ampicillin was inoculated with glycerol stock of *Escherichia coli* Dh5α cells carrying pBluescript II SK(+) (Stratagene) plasmid. The culture was incubated overnight at 37°C and shaken at 220 rpm. Plasmid DNA was isolated according to the manufacturer recommendations by using plasmid DNA isolation kit (K0502, Thermo Fisher Scientific™) and kept at -20°C until used.

DNA protective activity of both leaf and flower methanol extract of *F. cassia* were tested against the damage caused by hydroxyl radicals and UV radiation as previously described in the literature (Berk *et al*., 2011) with slight modifications. 5 µL plasmid DNA (˜25 ng/µL), 5 µL of 3% H_2O_2 and 5 µL of plant extract (100 mg/mL) of extract (dissolved in ddH_2O) were mixed and exposed to UV in the transilluminator (300 nm) for 5 min at room temperature. Only circular plasmid DNA without any agent that can cause DNA damage was used as the negative control. 6X loading dye (Thermo Scientific) was added to the mixture. The samples were run on a 0.8% agarose gel supplemented with 5 µL of 10 mg/mL ethidium bromide. The agarose gel electrophoresis was run at 90 V for 1 hour. The gel was visualized and photographed by using Quantum ST5 Gel Documentation system.

Results and Discussion

HPLC Analysis of phenolic compounds: The qualitative and quantitative analysis of 15 phenolic compounds from the flowers and leaves extracts of *F. cassia* were carried out by HPLC PDA (Table 3, Figs. 1 and 2). The amount of each compound was determined based on dry extract as mg/g. Galllic acid, 4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, salicylic acid, chicoric acid, cinnamic acid, epicatechin, rutin, apigenin 7-*O*-glucoside, naringenin and quercetin were identified by comparison to the retention times and UV spectra of authentic standards analyzed under identical analytical conditions. 15 phenolic compounds were found to be either high or low or not in methanol extracts of the flower and leaves.

According to our HPLC data, more phenolic compounds were identified in the methanol extract of the leaves of the species than flowers. However, the most abundant compound was determined as chicoric acid (159.754 mg/g) in the flower extract followed by chlorogenic acid (41.692 mg/g) and apigenin 7-*O*-glucoside

(6.747 mg/g). The predominant components of leaves extract have been found to be chicoric acid (107.754 mg/g) followed by chlorogenic acid (99.246 mg/g) and rutin (4.158 mg/g), respectively. It is seen that chlorogenic acid and chicoric acid are found to be much higher compounds in both flowers and leaves than other substances. While *p*-coumaric acid (0.316 mg/g) was the lowest compound in flower extract, epicatechin (0.151 mg/g) and gallic acid (0.088 mg/g) were the minor components in leaf extract of the species. However, vanillic acid, ferulic acid, salicylic acid, naringenin and quercetin could not be detected in both extracts. Chemical structures of the quantified compounds are shown in Figure 3.

Formerly, phytochemical content of *F. cassia* has been partially investigated in the literature (Karakaya *et al*., 2019; Bakar *et al*., 2021; Akalın & Koçyiğit, 2010). Coumarins including peucedanol suberosin, grandivitinol and umbelliferone were isolated from roots of the species using methanol (Karakaya *et al*., 2019). Also, two coumarin derivatives, felamedin and prantschimgin that are found to be present in ethanolic extracts of roots (Bakar *et al*., 2021). From the compounds identified herein, only quercetin has been previously reported to be present after acid hydrolysis in ethanol extract of leaves using thin layer chromatography (Akalın & Koçyiğit, 2010).

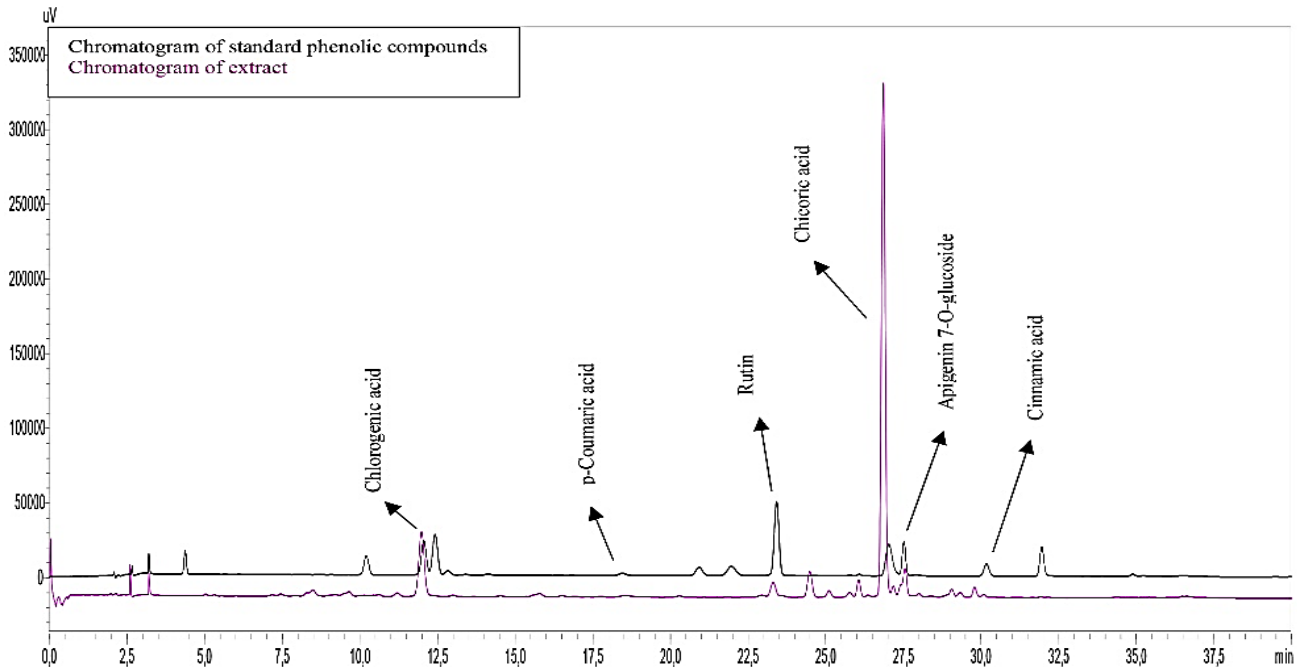

Fig. 1. HPLC chromatogram of standard phenolic compounds and methanol extract of flowers of *F. cassia.*

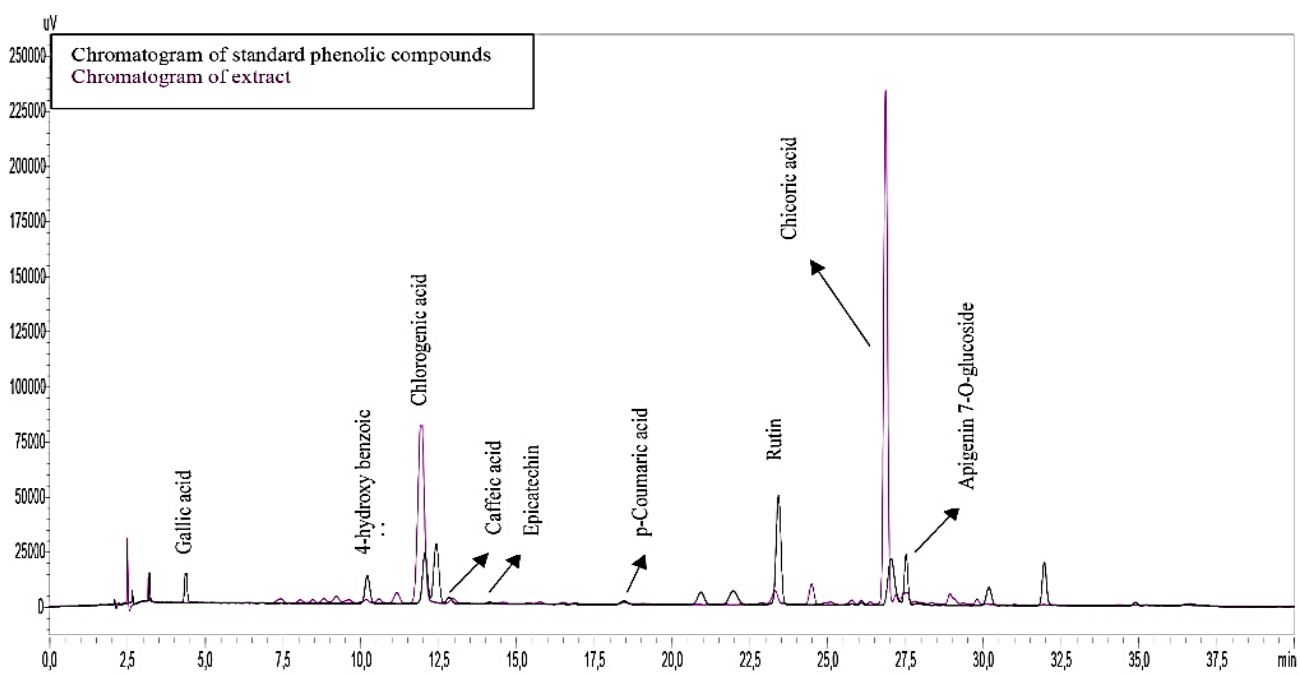

Fig. 2. HPLC chromatogram of standard phenolic compounds and methanol extract of leaves of *F. cassia.*

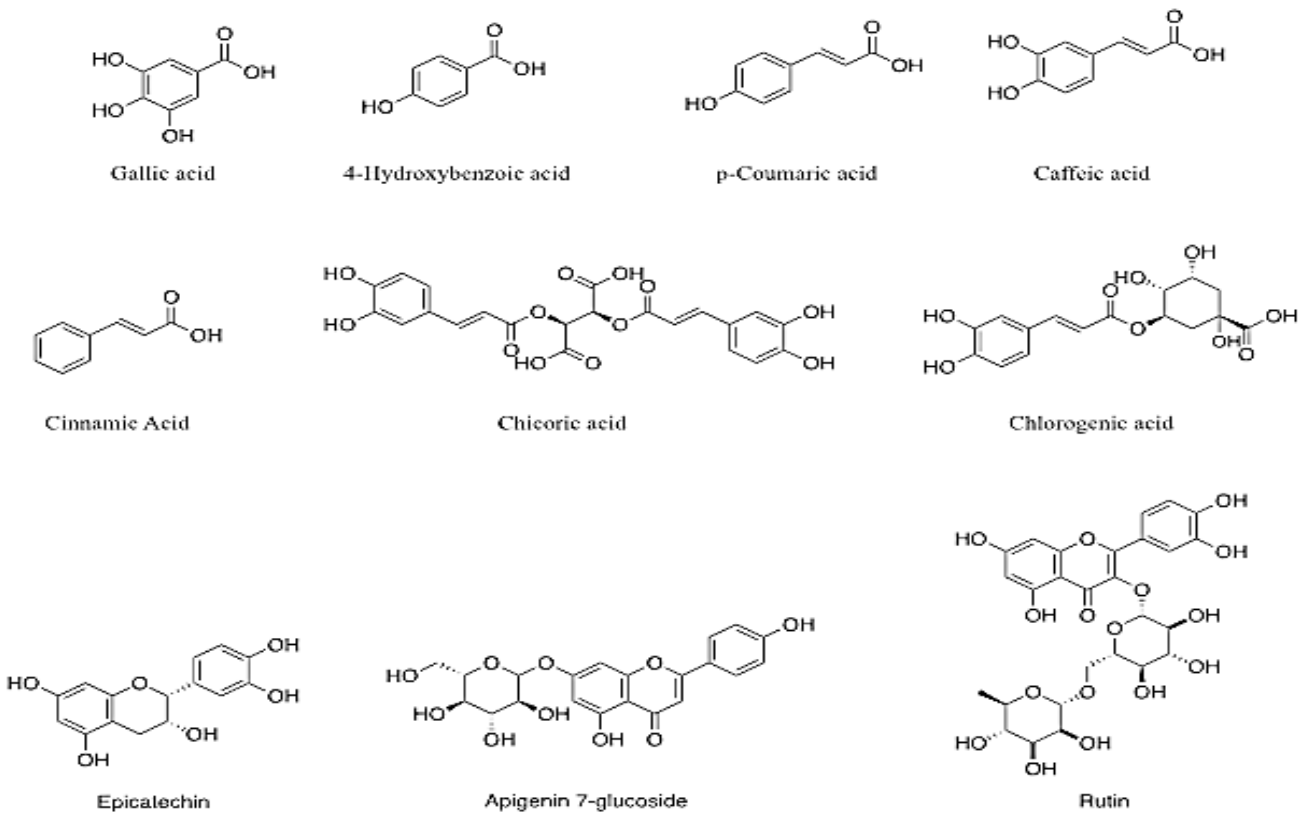

Fig. 3. Structures of identified phenolic compounds in flowers and leaves extracts of *F. cassia.*

Chemotaxonomic significance: From the chemotaxonomic point of view, some phenolic compounds found in *F. cassia* in this study have been detected previously from different parts of *Ferulago* species. Gallic acid and coumaric acid were reported in the stem and seeds of *F. angulata* (Hazrati *et al*., 2019). Chlorogenic acid was identified from the leaf and stems of *F. angulata* (Hazrati *et al*., 2019), from the aerial parts of *F. asparagifolia* (Alkhatib *et al*., 2009) and *F. stellata* (Kızıltaş *et al*., 2021) and *F. setifolia* (Zengin *et al*., 2020). Caffeic acid has also been reported in *F. setifolia* (Zengin *et al*., 2020) and *F. angulata* (Hazrati *et al*., 2019; Mousavi *et al*., 2022). Chicoric acid has been found in *F. angulata* (Hazrati *et al*., 2019; Mousavi *et al*., 2022). Cinnamic acid and epicatechin have been identified as components of *F. platycarpa* (Mohammed *et al*., 2020). Rutin is one of the most detected flavonoid glycosides in *Ferulago* species. It has been identified in the extracts of *F. asparagifolia* (Doğanca *et al*., 1992), aerial parts of *F. confusa* (Doğanca *et al*., 1995) and flowers of *F. campestris* (Maggio *et al*., 2013). Apigenin 7-*O*-glucoside has been reported in aerial parts of *F. setifolia* by Zengin *et al*., (Zengin *et al*., 2020).

The presence of phenolic compounds including vanillic acid, ferulic acid, salicylic acid, quercetin and naringenin that have not been reported in both leaves and flowers in this study. Thus, those results might be important in terms of chemotaxonomy. Vanillic acid and ferulic acid have been previously reported in flowers of *F. angulata* (Hazrati *et al*., 2019). Salicylic acid was identified in the aerial parts of *F. stellata* (Kızıltaş *et al*., 2021). Quercetin was found to be present in several *Ferulago* species including *F. asparagifolia* (Doğanca *et al*., 1992), *F. platycarpa* (Mohammed *et al*., 2020), *F. angulata* (Khanahmadi *et al*., 2011) and *F. setifolia* (Zengin *et al;* 2020). Moreover, Akalın *et al*., (Akalın and Koçyiğit, 2010) have reported several *Ferulago* species growing in Turkey including *F. trachycarpa*, *F. bracteata*, *F. syriaca*, *F. humulis* and *F. tojana* containing quercetin. However, in this study, the aglycones were liberated by breaking the glycosidic bonds using acidic hydrolysis. The structure of rutin, found in *F. cassia*, consists of an aglycone quercetin and a disaccharide rutinose. Thus, the occurrence of the quercetin might have formed as a result of the hydrolysis of rutin (Das *et al*., 2019). Lastly, naringenin has been also observed in aerial parts of *F. stellata* (Kızıltaş *et al*., 2021).

DNA Barcoding and phylogenetic tree: A ˜628 bp ITS sequence was obtained from the sequencing of the PCR product. Both forward and reverse reactions were sequenced and used for sequence confirmation. Contig was obtained from these forward and reverse sequences by using Bioedit (Hall, 1999). Blast analysis showed that this sequence was 96.6% similar to *F. macrocarpa*.1 (MK961982) as this is the first sequence record of *F. cassia*. Then the sequence was registered in GenBank with the accession number OM522013. The aligned matrix of nrDNA ITS data had 591 characters. The genus *Ferulago* forms a separate clade which *F. cassia* OM522013 located in. *F. cassia* nested more closely to *F. macrocarpa* MK961982.1 and all these species form a strongly supported group in a similar fashion as shown in previous studies (Downie *et al*., 2010; Lyskov *et al*., 2015) (Fig. 4). This is the first sequence data of *F. cassia* and this result proves that this plant material is *F. cassia* without any doubt.

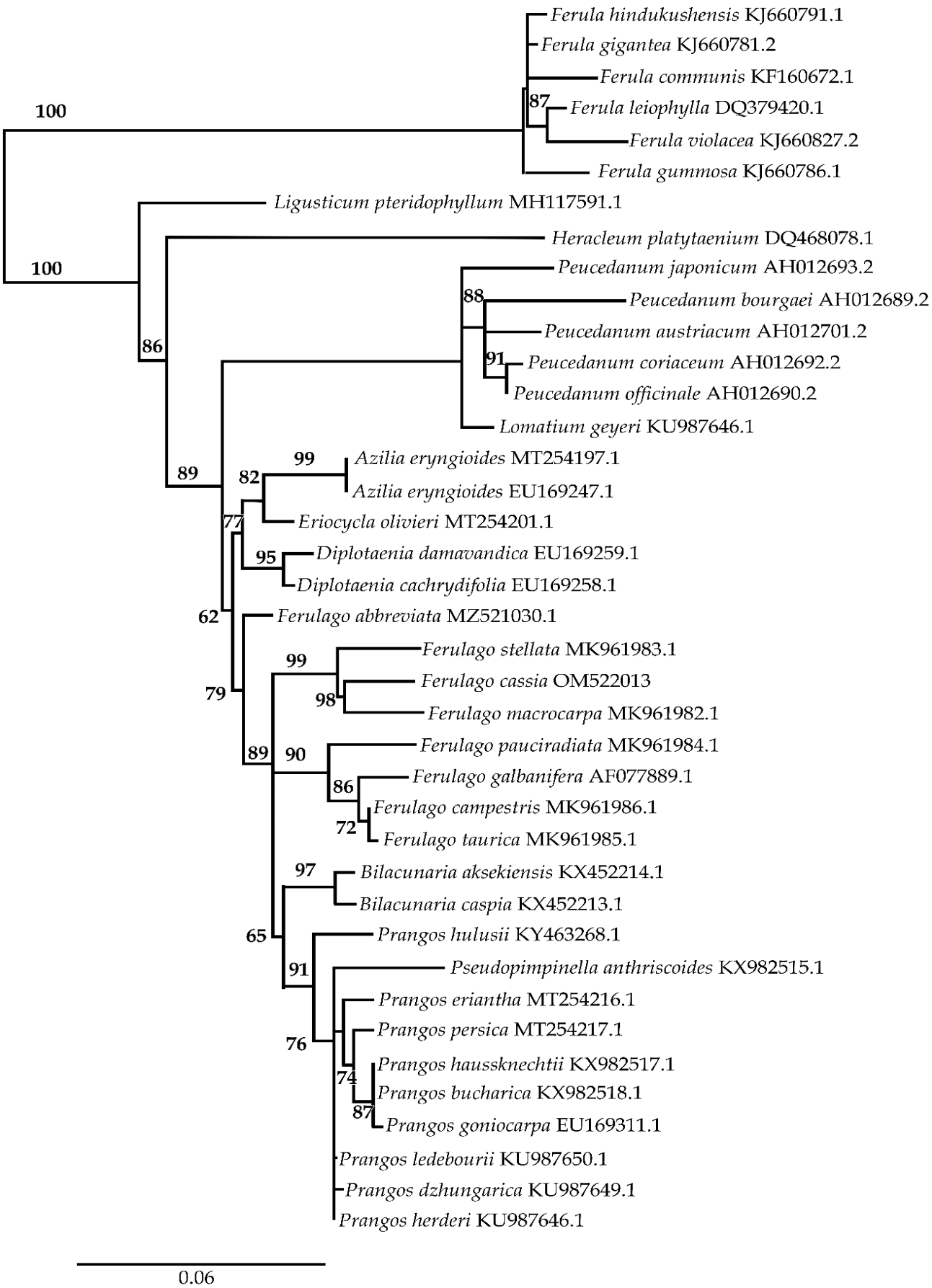

Fig. 4. NJ phylogenetic tree of the nuclear ribosomal DNA ITS sequences of the studied taxa. Bootstrap percentages appear above the branches. Values higher than 50 % are shown on the corresponding branches. The Genebank accession numbers of the reference sequences were mentioned in figure.

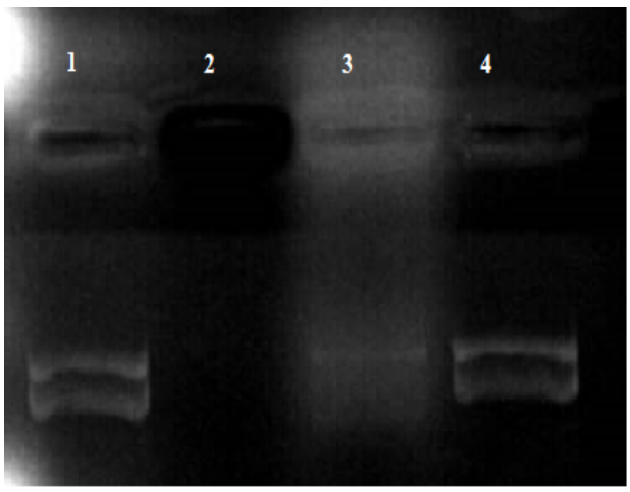

Fig. 5. DNA Protective activity of *F. cassia* methanol extract. 1: Circular plasmid DNA, 2: plasmid DNA + 3% H_2O_2 +UV (5 min.), 3: plasmid DNA + 3% H_2O_2 +UV (5 min.) + *F. cassia* leaf methanol extract, 4: plasmid DNA + 3% H_2O_2 +UV (5 min.) + *F. cassia* flower methanol extract.

DNA Protective activity: Photolysis of H_2O_2 by UV causes the formation of OH radicals (Guha *et al*., 2011). DNA scissions have shown to be an effective method to evaluate the antioxidant activity against hydroxyl radicals which lead to oxidative damage, causes carcinogenesis, mutagenesis and cytotoxicity, *In vitro* (Kumar *et al*., 2014). Previous studies showed that *F. syriaca*, *F. longistylis*, *F. isaurica*, *F. setifolia* and *F. cassia* inhibits cell viability in PC3 and SW480 cells (Bakar *et al*., 2021) and aerial parts of *F. mughlae* has anticancer activity on human prostate (PC-3) and colorectal (SW-480) carcinoma cells (Bakar *et al*., 2016). In addition; *Coriandrum sativum* L. (Harsha & Anilakumar, 2014), *Trachyspermum ammi* L. and *Foeniculum vulgare* Mill. (Goswami & Chatterjee, 2014) showed similar DNA protective activity results that all belong to Apiaceae family against hydroxyl radicals. When DNA damage of occurs in one of the phosphodiester chains of a circular plasmid, the cleavage of the supercoiled DNA produces a relaxed open circular form. They can produce OH radicals. In this study, *In vitro* DNA damage protection of the methanol extract of *F. cassia* against H_2O_2 and UV was evaluated. Addition of H_2O_2 and UV decreases the presence of supercoiled DNA. As clearly seen in Figure 5, both plant extracts offer DNA protection with a slight difference of flower extract having a little bit more protection. The addition of both extracts increased the visibility of the plasmid DNA which disappeared after H_2O_2 and UV stress. When this type of action occurs, the flower and leaf methanol extracts of *F. cassia* might diminish the reduction potential of H_2O_2 and UV, and might also directly protecting the supercoiled plasmid DNA from OH radical dependent strand breaks as the taxa belonged to Apiaceae so as *Ferulago* species have potent anticancer effects and are known as an Important source of antioxidants (Bakar *et al*., 2021; Kumari *et al*., 2011; Thiviya *et al*., 2021). *F. cassia* methanol extract might prevent the reaction with UV and H_2O_2. On the other hand, natural polyphenols can influence the oxidation of DNA through simple mechanisms including quenching of reactive oxygen species by donating hydrogen atoms or electrons (Singh *et al*., 2009). As the genus *Ferulago* is a rich natural source for phenolic contents and also coumarins and phytosteroids, previous results of *F. cassia* extracts showed the genus has promising potential anticancer agents against treated cancer cell lines due to coumarins and phytosteroids (Bakar *et al*., 2021; Mohammed *et al*., 2020). It can be thought that chlorogenic acid and chicoric acid, which are the major components in both flowers and leaves, may be responsible for this effect. Chicoric acid has been shown to provide UV protection better than the total of the substrates in purple coneflower plants (Fu *et al*., 2021) and its DNA protective activity was studied by Liu *et al*., (Liu *et al*., 2017). Besides, DNA protective activity of chlorogenic acid was also proved (Xu *et al*., 2012). According to our results, chicoric and chlorogenic acids are the major compounds of both of the leaves and flowers methanol extracts of *F. cassia*. The sum of the both compounds in flowers extract is 201,446 mg/g and in leaves extract is 207 mg/g. The difference of DNA protective activity between *F. cassia* leaves and flowers methanol extracts may be from the presence of other compounds in the flowers methanol extract as both of the compounds proved to protect DNA against oxidative damage (Cinkilic *et al*., 2014; Zheng *et al*., 2005). This result may also be a proof that *F. cassia* extracts will be a potential anticancer agent.

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Supplementary Material Ferulago cassia flower extract chromatograms.

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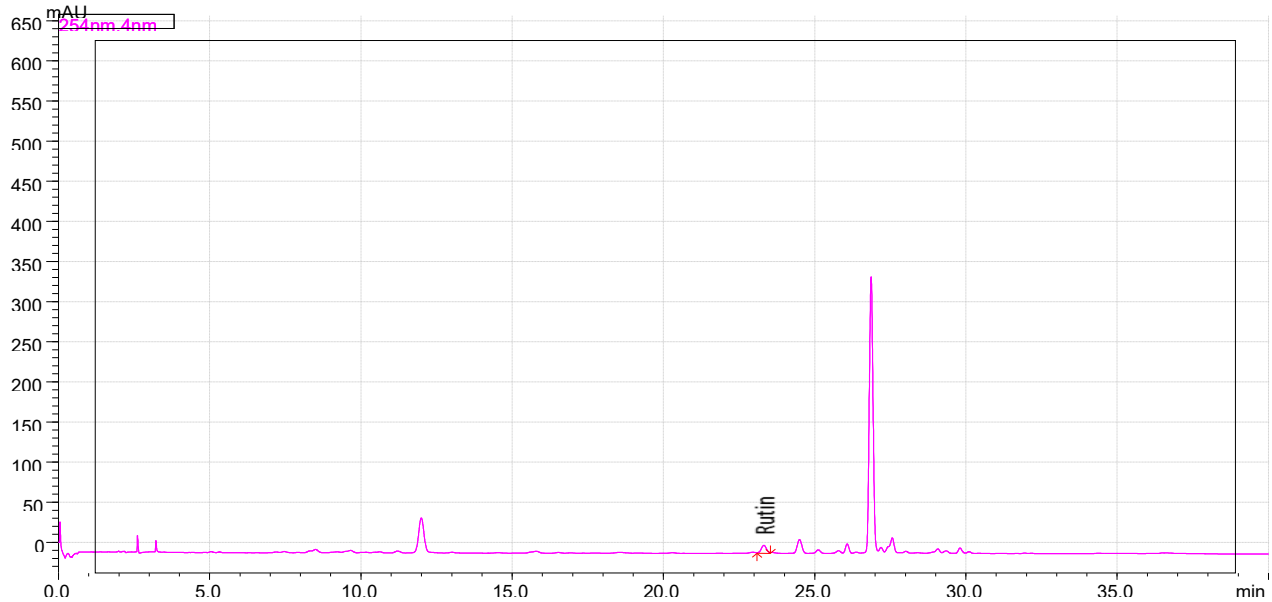

Fig. S1. HPLC chromatogram of rutin.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	23.320	121959	9879		6.003	mg/g	10	Rutin	100.000	1.19
Total		121959	9879		6.003				100.000	

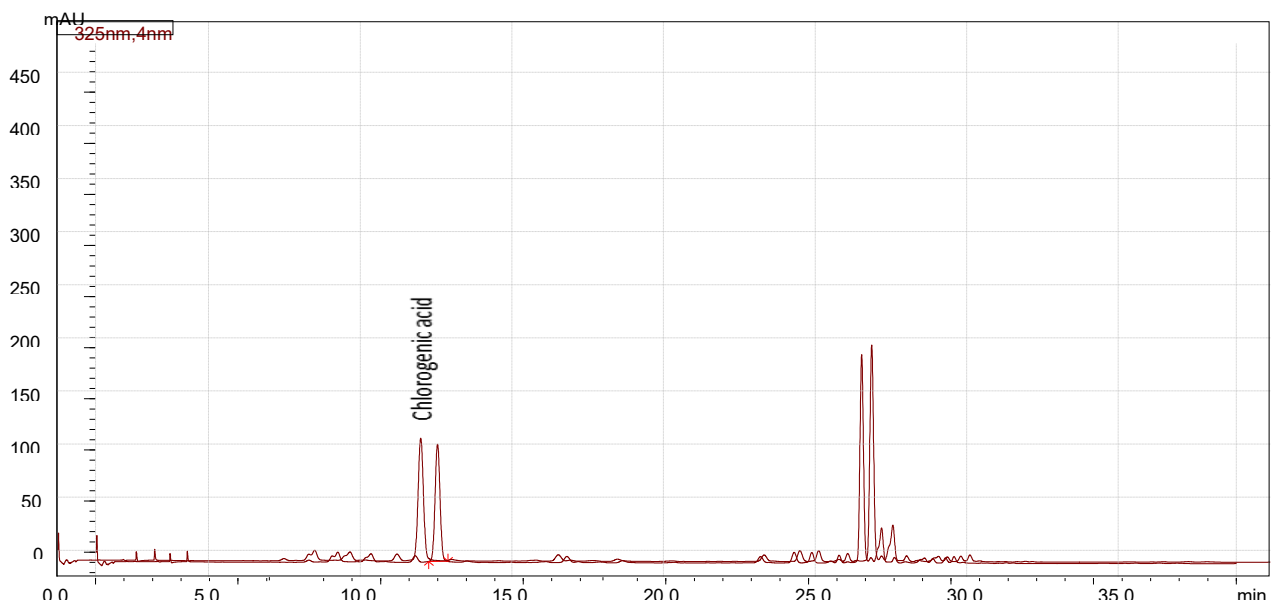

Fig. S2. HPLC chromatogram of chlorogenic acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	11.994	1389074	114297		41.692	mg/g	3	Chlorogenic acid	100.000	17.58
Total		1389074	114297		41.692				100.000	

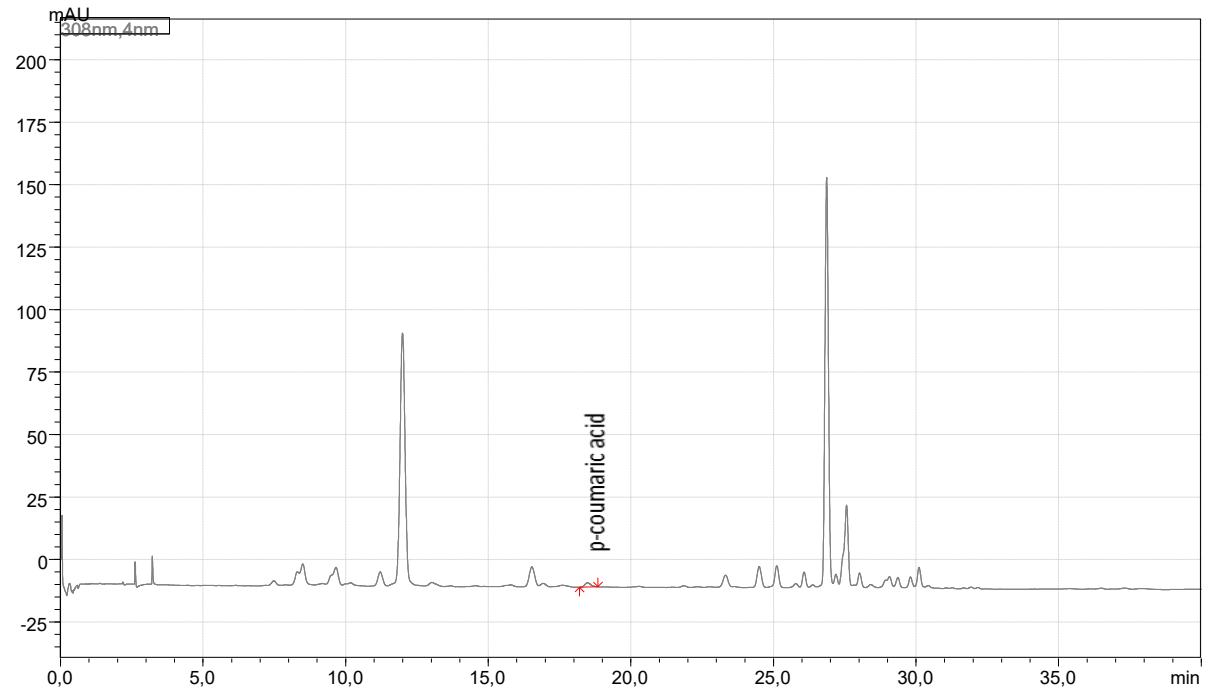

Fig. S3. HPLC chromatogram of p-coumaric acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	18.482	25011	1712		0.316	mg/g	7	p-Coumaric acid	100.000	0.24
Total		25011	1712		0.316				100.000	

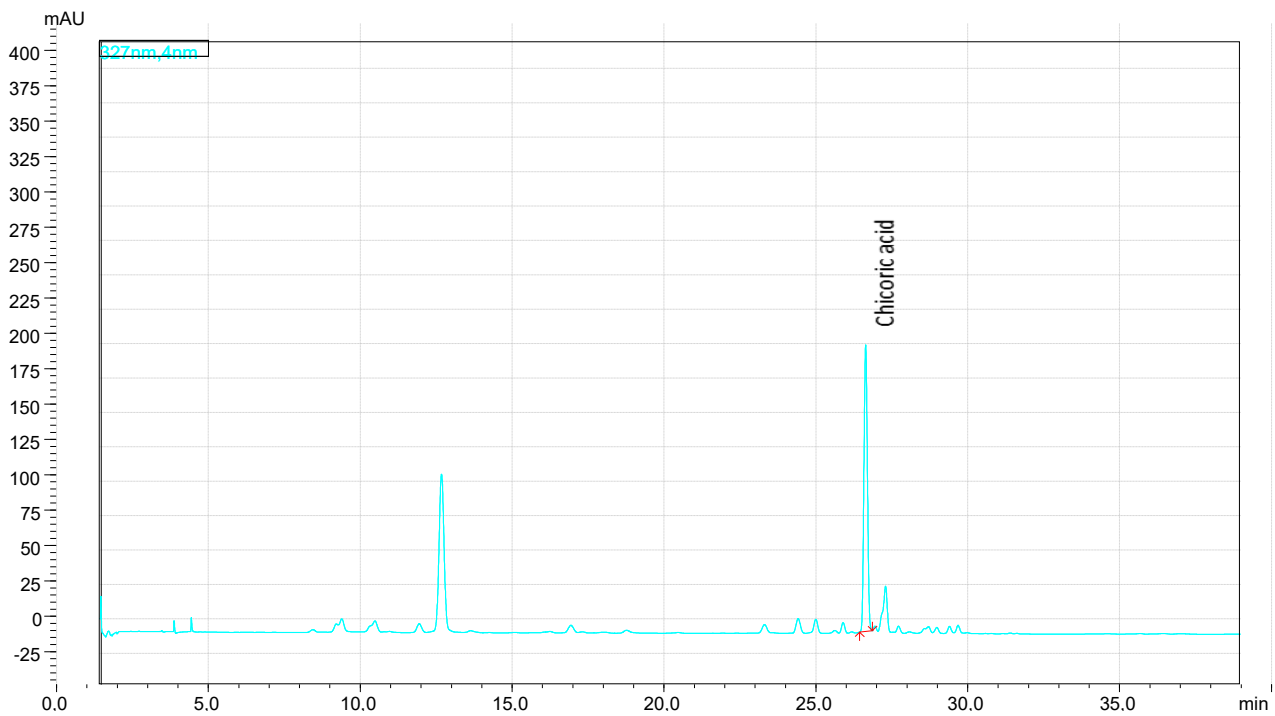

Fig. S4. HPLC chromatogram of chicoric acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	26.866	1770443	207650		159.754	mg/g	11	Chicoric acid	100.000	48.32
Total		1770443	207650		159.754				100.000	

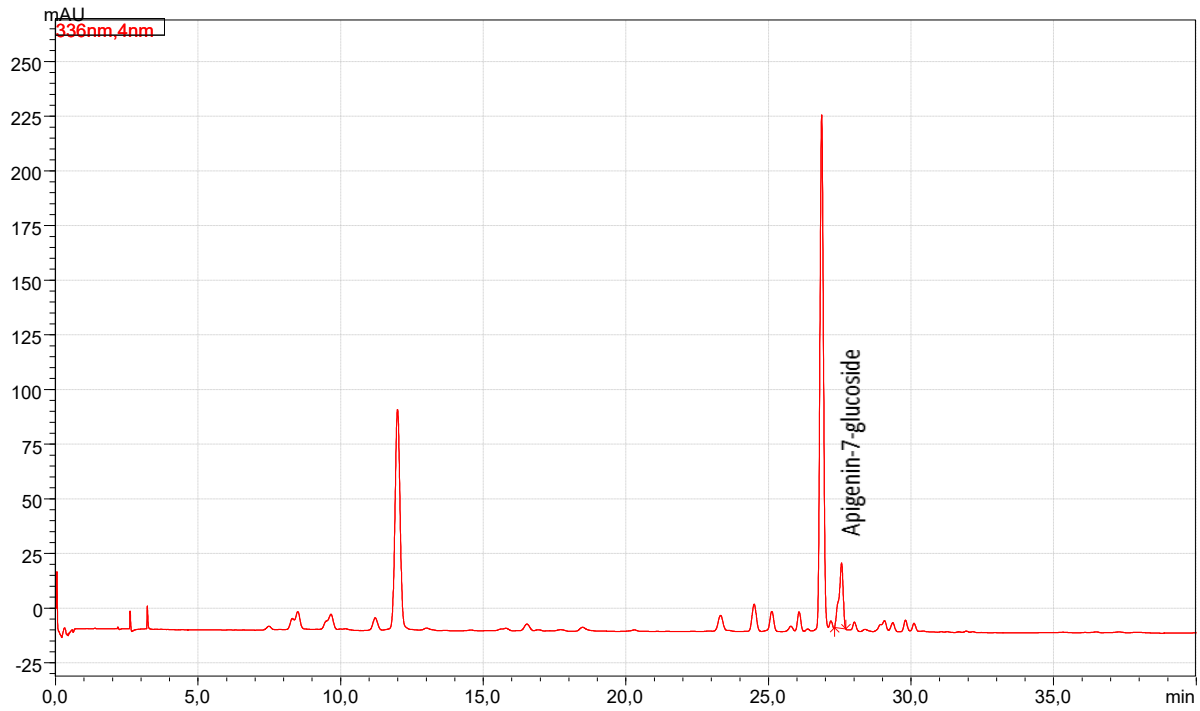

Fig. S5. HPLC chromatogram of apigenin-7-glucoside.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	27.563	307722	29869		6.747	mg/g	12	Apigenin-7-*O*-glucoside	100.000	4.30
Total		307722	29869		6.747				100.000	

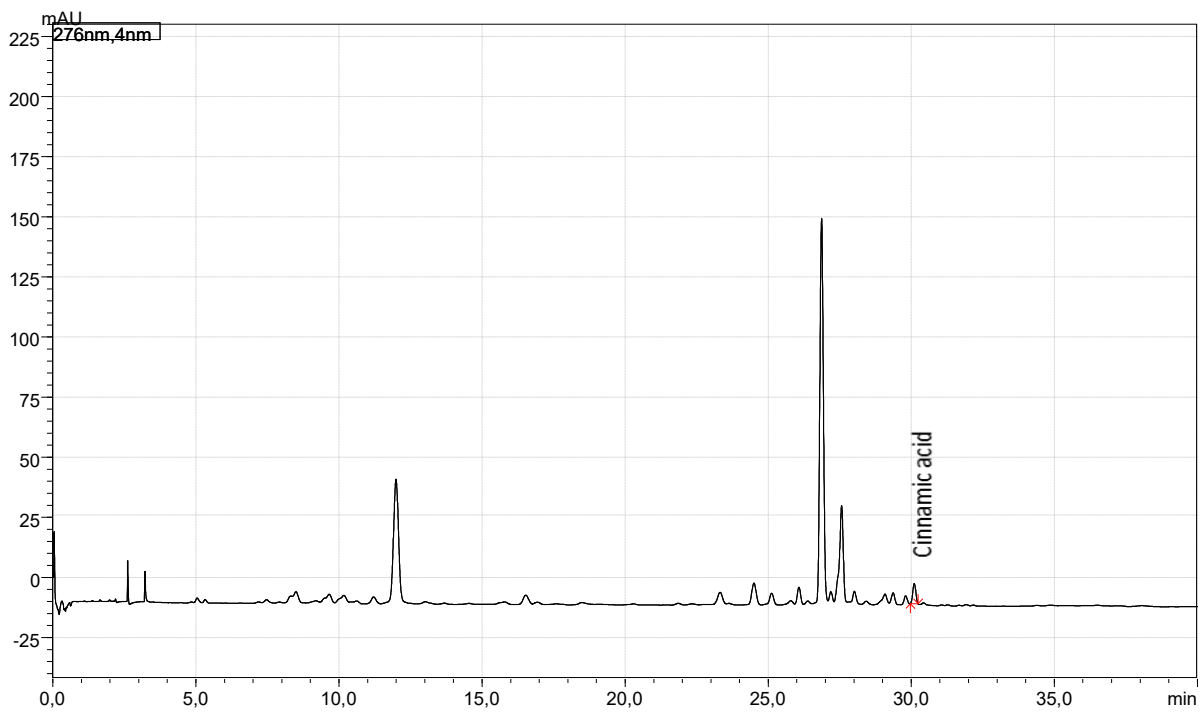

Fig. S6. HPLC chromatogram of cinnamic acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	30.105	62834	8360		0.705	mg/g	13	Cinnamic acid	100.000	1.52
Total		62834	8360		0.705				100.000	

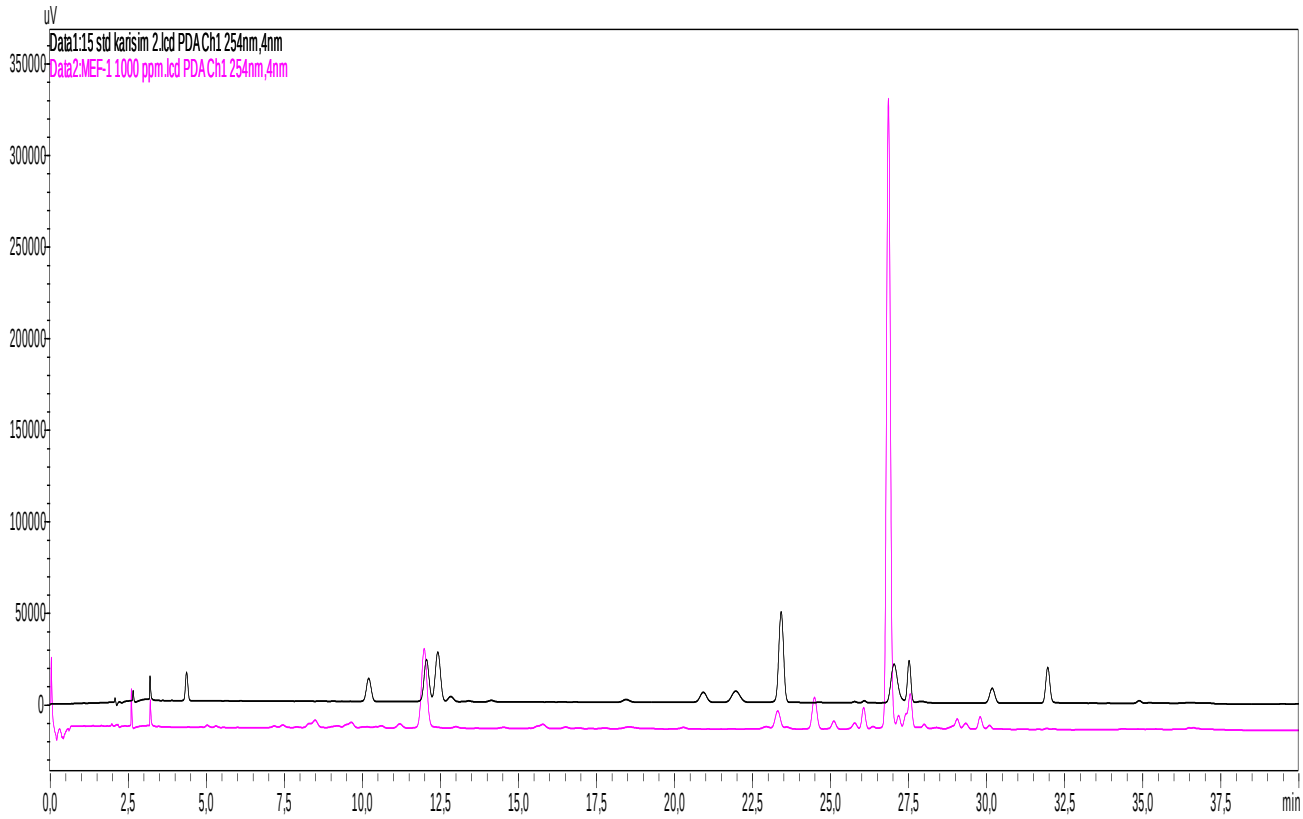

Fig. S7. HPLC chromatogram of *Ferulago cassia* flower extract.

ID#	Name	Ret. Time	Conc.	Unit	Channel	Peak#	Area	Height	Area%	S/N
1.	Gallic acid	No peak is detected.	0.000	mg/g	Ch2 271nm	--	0	0	0.000	--
2.	4-Hydroxybenzoic acid	No peak is detected.	0.000	mg/g	Ch1 254nm	--	0	0	0.000	--
3.	Chlorogenic acid	11.994	41.692	mg/g	Ch3 325nm	1	1389074	114297	100.000	17.58
4.	Vanillic acid	No peak is detected.	0.000	mg/g	Ch4 260nm	--	0	0	0.000	--
5.	Caffeic acid	No peak is detected.	0.000	mg/g	Ch5 248nm	--	0	0	0.000	--
6.	Epicatechin	No peak is detected.	0.000	mg/g	Ch6 277nm	--	0	0	0.000	--
7.	p-Coumaric acid	18.482	0.316	mg/g	Ch7 308nm	1	25011	1712	100.000	0.24
8.	Ferulic acid	No peak is detected.	0.000	mg/g	Ch8 322nm	--	0	0	0.000	--
9.	Salicylic acid	No peak is detected.	0.000	mg/g	Ch9 235nm	--	0	0	0.000	--
10.	Rutin	23.320	6.003	mg/g	Ch1 254nm	1	121959	9879	100.000	1.19
11.	Chicoric acid	26.866	159.754	mg/g	Ch10 327nm	1	1770443	207650	100.000	48.32
12.	Apigenin-7-*O*-glucoside	27.563	6.747	mg/g	Ch11 336nm	1	307722	29869	100.000	4.30
13.	Cinnamic acid	30.105	0.705	mg/g	Ch12 276nm	1	62834	8360	100.000	1.52
14.	Quercetin	No peak is detected.	0.000	mg/g	Ch1 254nm	--	0	0	0.000	--
15.	Naringenin	No peak is detected.	0.000	mg/g	Ch13 288nm	--	0	0	0.000	--

Supplementary Material Ferulago cassia leaf extract chromatograms.

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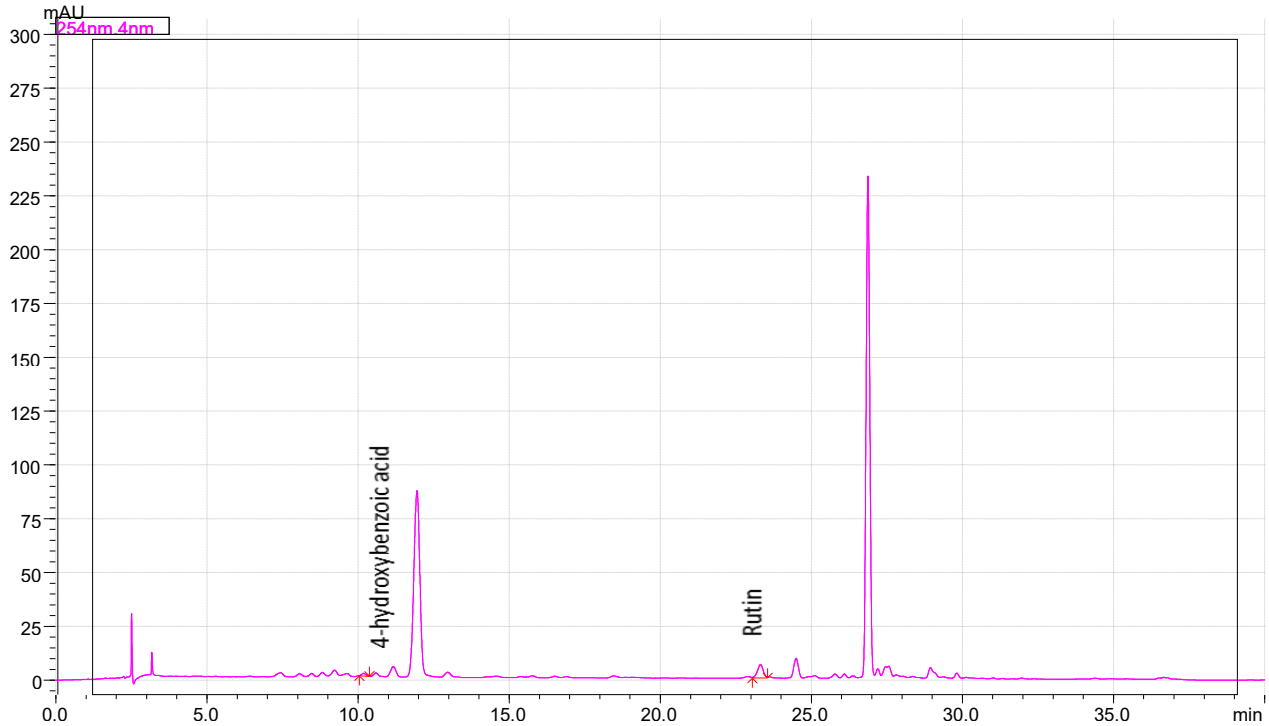

Fig. S1. HPLC chromatogram of 4-hydroxybenzoic acid and rutin.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	10.184	10897	1228		0.166	mg/g	2	4-Hydroxybenzoic acid	11.427	0.20
2	23.317	84467	6226		4.158	mg/g	10	Rutin	88.573	1.00
Total		95364	7454		4.324				100.000	

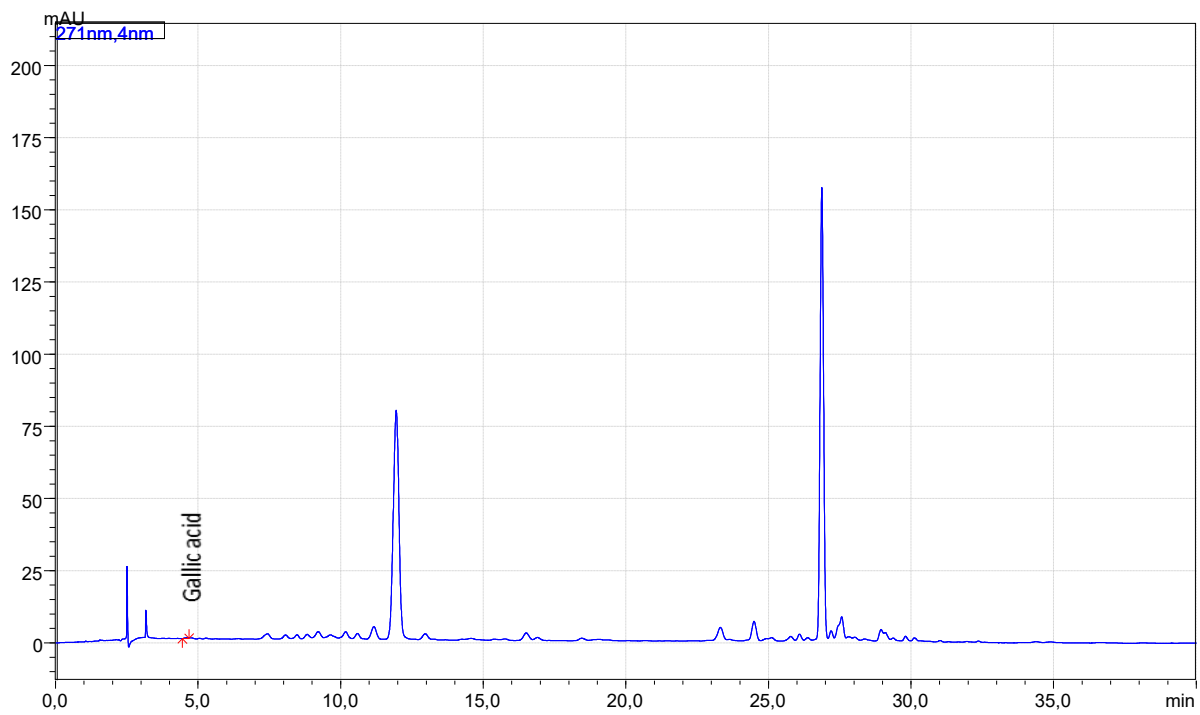

Fig. S2. HPLC chromatogram of gallic acid.

Peak #	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	4.601	2703	273		0.088	mg/g	1	Gallic acid	100.000	0.06
Total		2703	273		0.088				100.000	

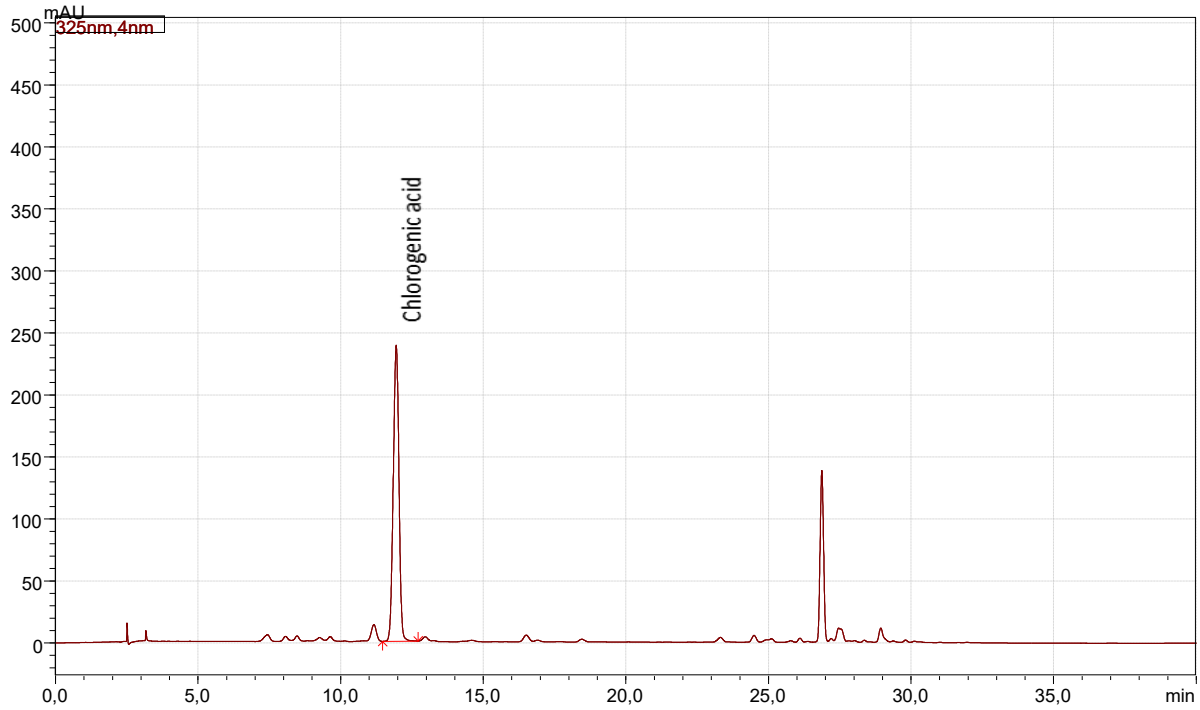

Fig. S3. HPLC chromatogram of chlorogenic acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	11.949	3306656	238547		99.246	mg/g	3	Chlorogenic acid	100.000	65.24
Total		3306656	238547		99.246				100.000	

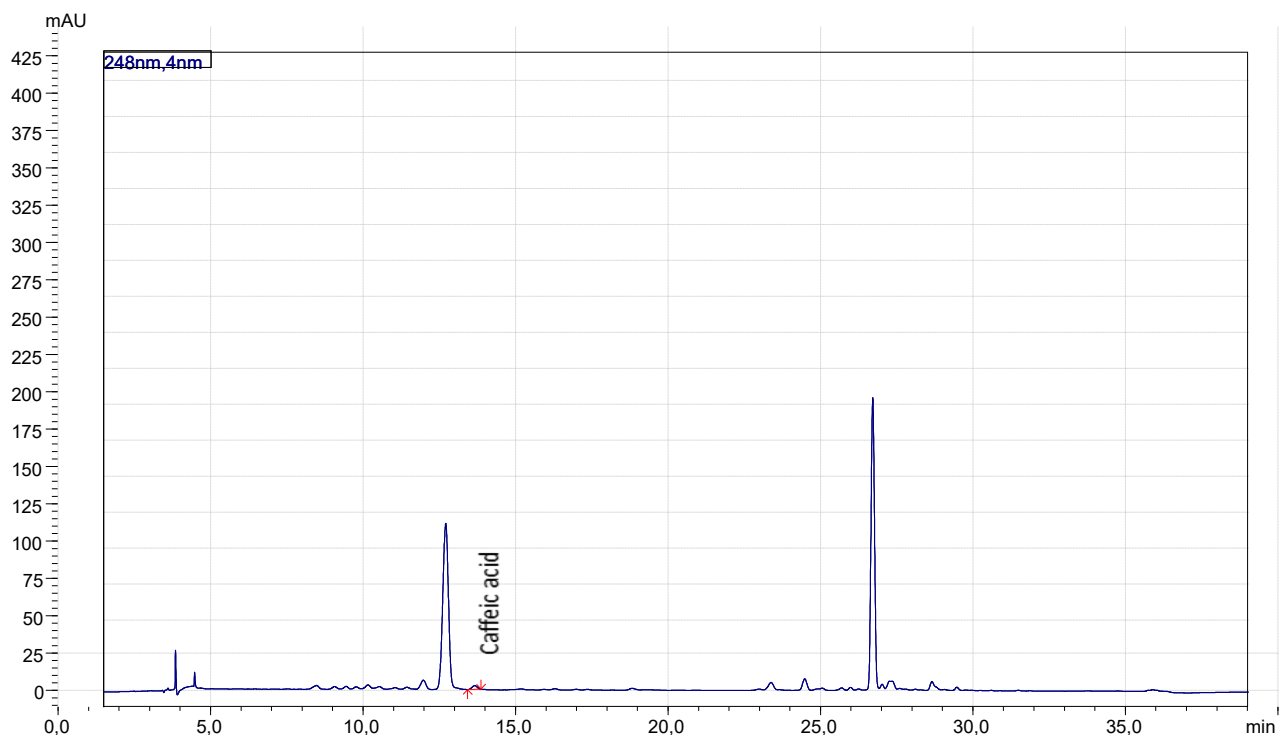

Fig. S4. HPLC chromatogram of caffeic acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	12.964	32652	2661		1.706	mg/g	5	Caffeic acid	100.000	0.43
Total		32652	2661		1.706				100.000	

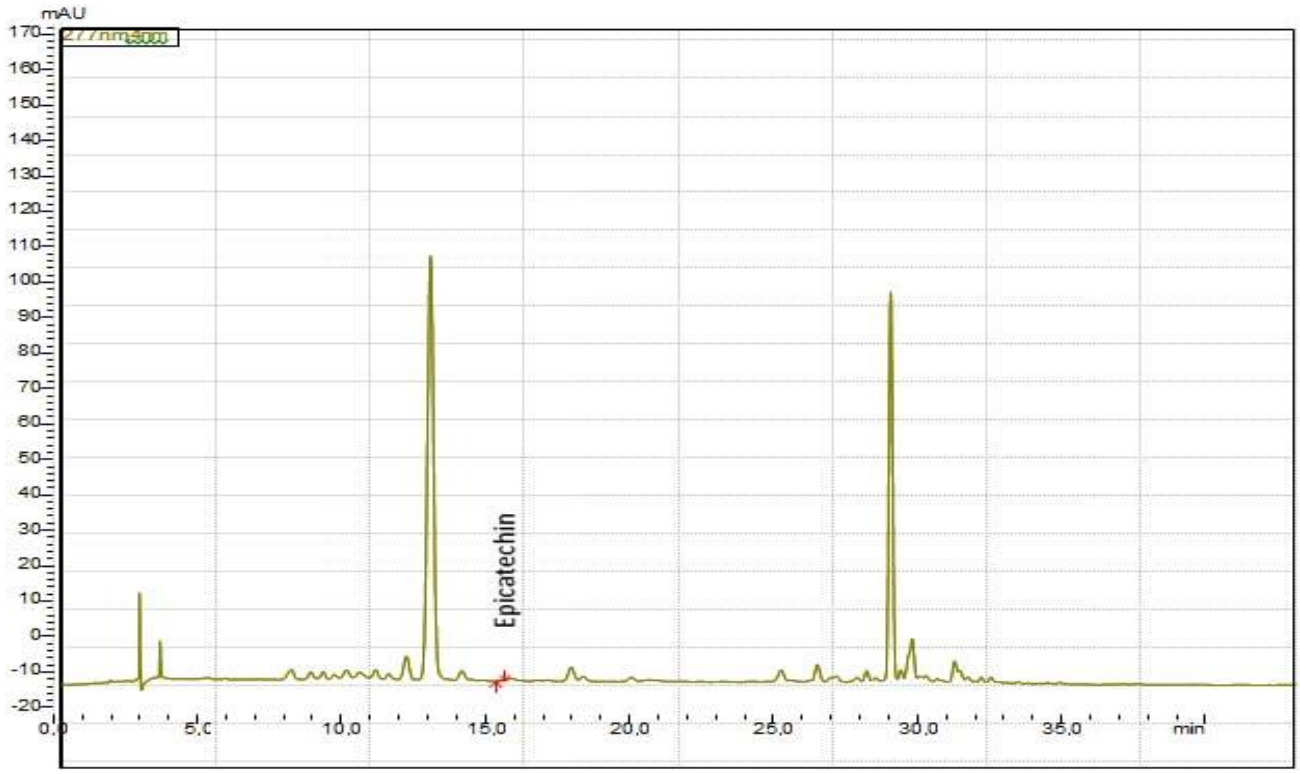

Fig. S5. HPLC chromatogram of epicatechin.

Peak #	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	14.232	1081	128		0.151	mg/g	6	Epicatechin	100.000	0.03
Total		1081	128		0.151				100.000	

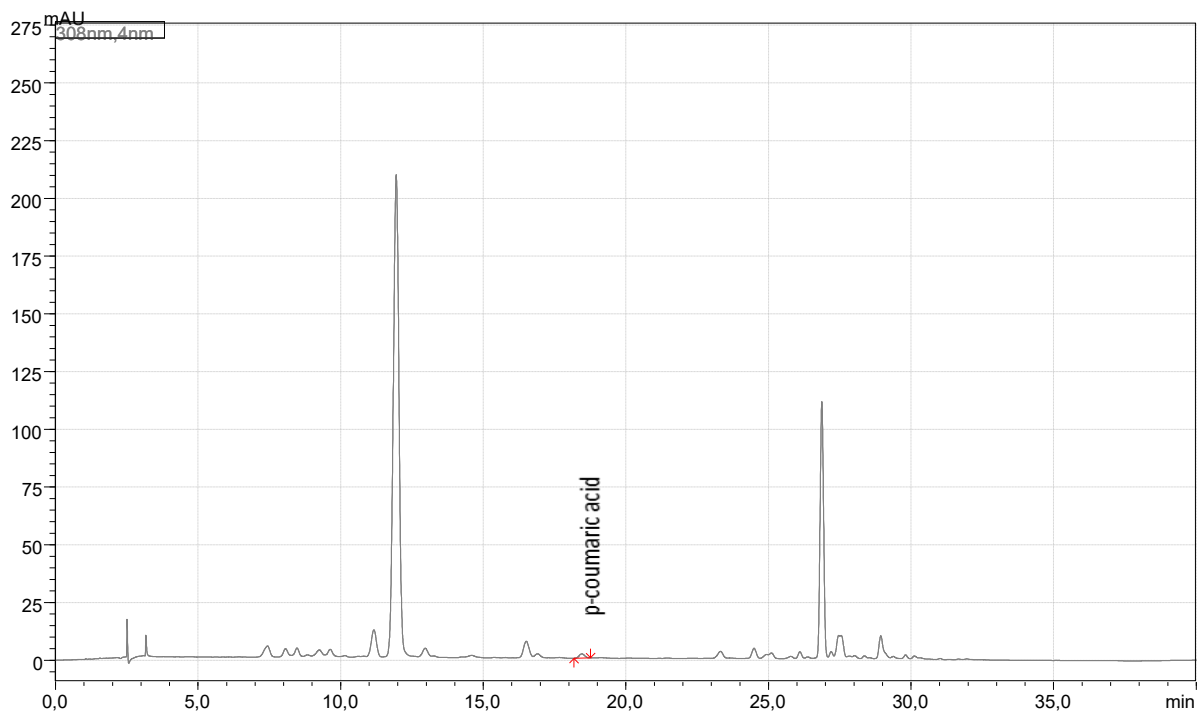

Fig. S6. HPLC chromatogram of p-coumaric acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	18.456	24857	1853		0.314	mg/g	7	p-Coumaric acid	100.000	0.30
Total		24857	1853		0.314				100.000	

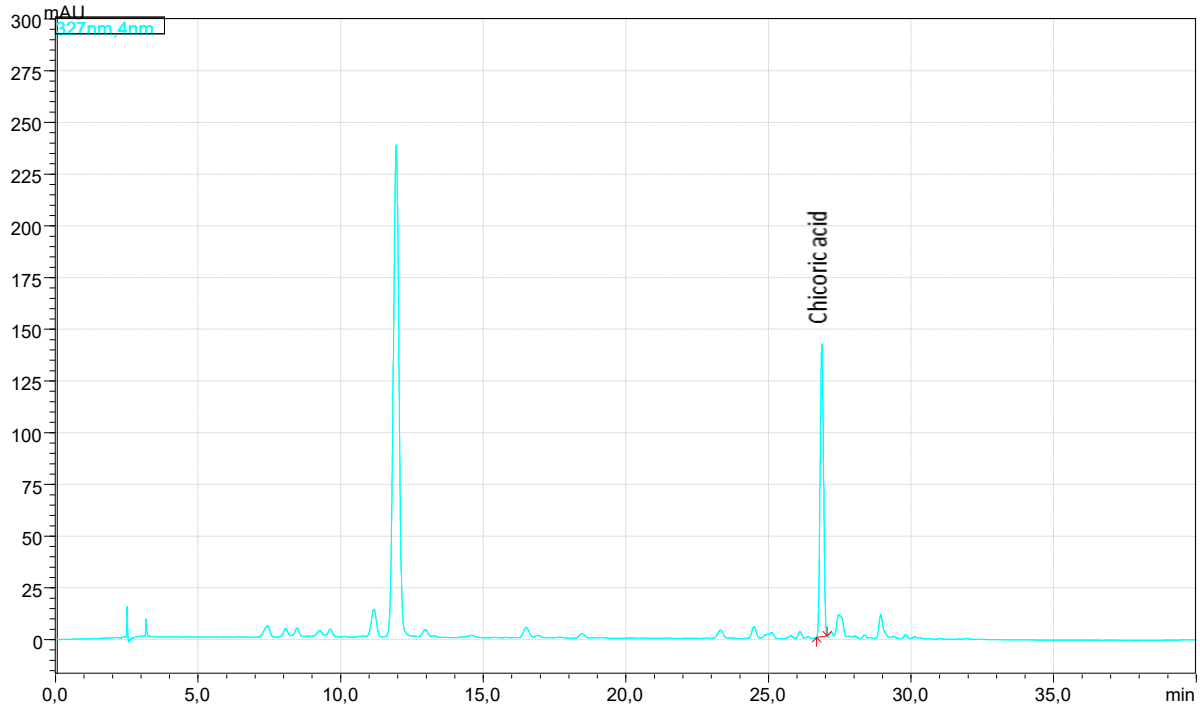

Fig. S7. HPLC chromatogram of chicoric acid.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	26.872	1194156	141288		107.754	mg/g	11	Chicoric acid	100.000	27.91
Total		1194156	141288		107.754				100.000	

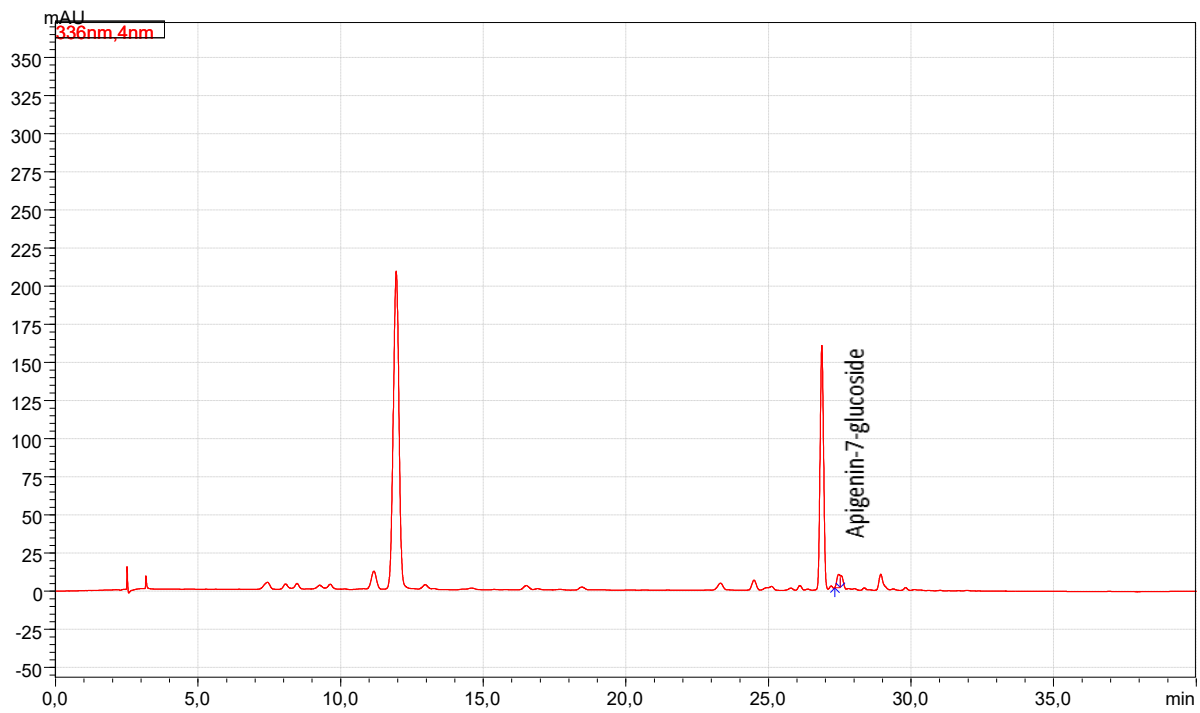

Fig. S8. HPLC chromatogram of apigenin-7-glucoside.

Peak#	Ret. Time	Area	Height	Mark	Conc.	Unit	ID#	Name	Area%	S/N
1	27.462	65313	8744		1.432	mg/g	11	Apigenin-7-*O*-glucoside	100.000	1.31
Total		65313	8744		1.432				100.000	

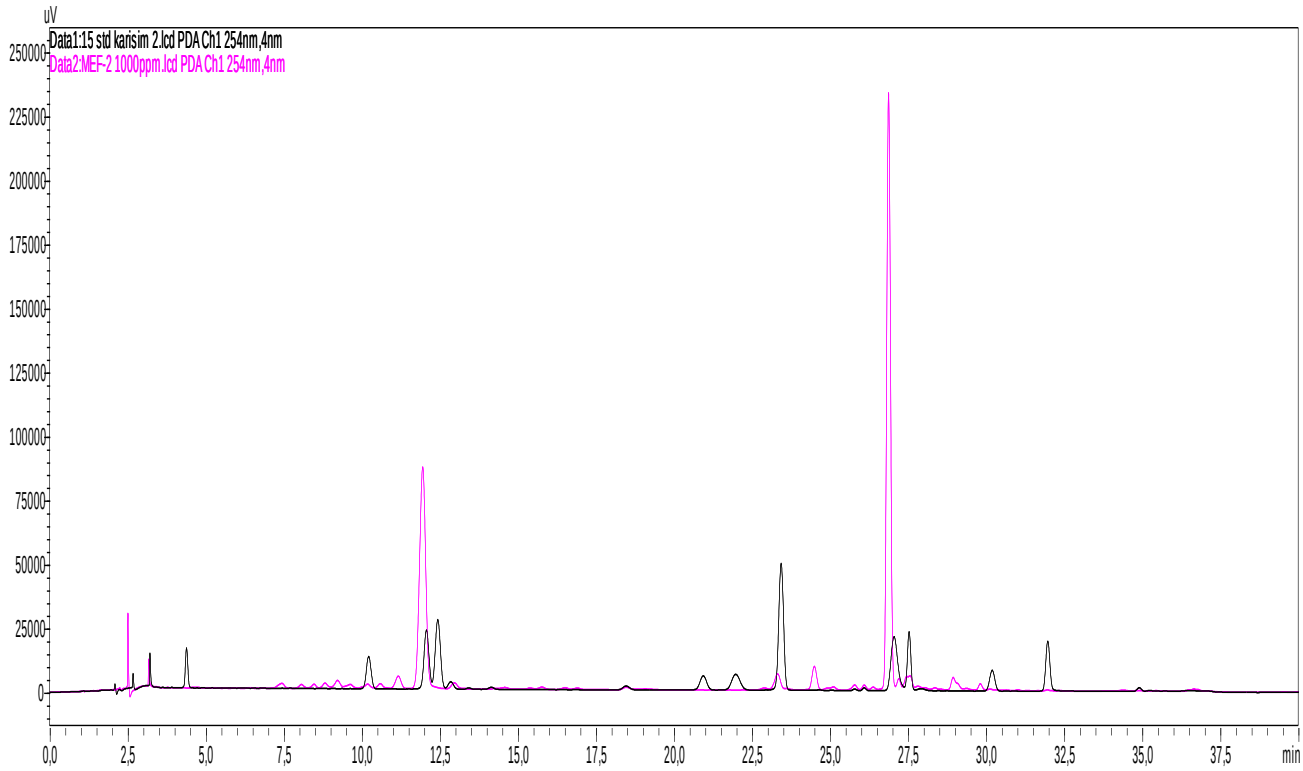

Fig. S9. HPLC chromatogram of *Ferulago cassia* leaf extract.

ID#	Name	Ret. Time	Conc.	Unit	Channel	Peak#	Area	Height	Area%	S/N
1.	Gallic acid	4.601	0.088	mg/g	Ch2 271nm	1	2703	273	100.000	0.06
2.	4-Hydroxybenzoic acid	10.184	0.166	mg/g	Ch1 254nm	1	10897	1228	11.427	0.20
3.	Chlorogenic acid	11.949	99.246	mg/g	Ch3 325nm	1	3306656	238547	100.000	65.24
4.	Vanillic acid	No peak is detected	0.000	mg/g	Ch4 260nm	--	0	0	0.000	--
5.	Caffeic acid	12.964	1.706	mg/g	Ch5 248nm	1	32652	2661	100.000	0.43
6.	Epicatechin	14.232	0.151	mg/g	Ch6 277nm	1	1081	128	100.000	0.03
7.	p-Coumaric acid	18.456	0.314	mg/g	Ch7 308nm	1	24857	1853	100.000	0.30
8.	Ferulic acid	No peak is detected	0.000	mg/g	Ch8 322nm	--	0	0	0.000	--
9.	Salicylic acid	No peak is detected	0.000	mg/g	Ch9 235nm	--	0	0	0.000	--
10.	Rutin	23.317	4.158	mg/g	Ch1 254nm	2	84467	6226	88.573	1.00
11.	Chicoric acid	26.872	107.754	mg/g	Ch10 327nm	1	1194156	141288	100.000	27.91
12.	Apigenin-7-*O*-glucoside	27.462	1.432	mg/g	Ch11 336nm	1	65313	8744	100.000	1.31
13.	Cinnamic acid	No peak is detected	0.000	mg/g	Ch12 276nm	--	0	0	0.000	--
14.	Quercetin	No peak is detected	0.000	mg/g	Ch1 254nm	--	0	0	0.000	--
15.	Naringenin	No peak is detected	0.000	mg/g	Ch13 288nm	--	0	0	0.000	--