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Comparative qualitative and quantitative determination of flavonoids and phenolic contents of *Cinnamomum verum* J. Presl and *Cinnamomum camphora* L. Growing in Egypt

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Abstract

The present study aims to compare qualitatively and quantitatively the flavonoids and phenolic contents of the leaves of *Cinnamomum verum* J. Presl and *Cinnamomum camphora* L. Growing in Egypt by established methods. UV-visible spectroscopy was employed to quantify the total flavonoids and total phenolics contents. The qualitative and quantitative estimation of the flavonoid and phenolic acid contents in the leaves of *Cinnamomum verum* J. Presl and *Cinnamomum camphora* L. were performed by High performance liquid chromatography (HPLC). The content of total phenolics of different *Cinnamomum* leaf extracts was (20.61 and 19.65 mg/100 g), respectively. While, the composition of total flavonoids of the leaves of *Cinnamomum verum* J. Presl and *Cinnamomum camphora* L. were (2.01 and 3.88 mg/100 g). The HPLC analysis of the flavonoid contents revealed the presence of eleven compounds in the leaf of *Cinnamomum camphora* L. with rutin as a major compound (5309.6 ppm) followed by quercetrin (6025.5 ppm) and nine compounds were estimated in the leaf of *Cinnamomum verum* J. Presl with rosmarinic as a major compound (6194 ppm) then Naringin (2281.3 ppm).

1. Introduction

Cinnamomum verum J. Presl and *Cinnamomum camphora* L. are significant medicinal plants within the *Lauraceae* family, widely utilized in herbal medicine. Species from this family hold considerable economic value due to their diverse applications, ranging from culinary spices to therapeutic agents [1]. Cinnamon, a key flavoring component in the food and beverage sector, has been esteemed since ancient times for both its aromatic and medicinal qualities[2]. The genus *Cinnamomum*, encompassing more than 250 species, thrives in tropical and subtropical climates. *Cinnamomum verum* J.Presl is particularly valued as a spice,

Vol. (2), No. (2), April-July; 2025. DOI:10.21608/MERMJ.2025.402345.1028

primarily due to its high cinnamaldehyde content[3]. Research has demonstrated that *Cinnamomum verum* J.Presl exhibits potent anti-inflammatory, antioxidant, anticancer, and antibacterial activities [4].

Conversely, *Cinnamomum camphora* L. has been traditionally employed in treating inflammation-related ailments, including rheumatism, sprains, bronchitis, and asthma, owing to its camphor content. The wood and leaves of this species possess analgesic, antispasmodic, odontalgic, and rubefacient properties, in addition to being used for digestive disorders, depression, and as a stimulant. Furthermore, infusions derived from *C. camphora* are inhaled to alleviate colds and pulmonary conditions[5]. Given their extensive pharmacological significance, a comparative quantitative and qualitative analysis of flavonoid and phenolic compounds in the leaves of *Cinnamomum verum* J. Presl and *Cinnamomum camphora* L. was conducted.

2. Material and Methods

2.1. Plant Material

Samples of the two leaves of *C. verum* J. Presl and *C. camphora* L. were collected in May to July from trees growing in Giza Zoo, Egypt. The plants were authenticated by Dr. Abdel Halim Mohammed, Senior Taxonomist. A voucher specimen, kept in the herbarium of the Pharmacognosy Department at Nahda University with codes CV-016 and CM-018 of *C. verum* J. Presl and *C. camphora* L. respectively.

2.2. Materials and Reagents

The reagents and chemicals required for UV spectroscopy were prepared following established methods outlined in reference [5]. The materials used for the UV spectroscopic analysis of flavonoids were sourced from E. Merck, located in Darmstadt, Germany.

Additionally, high-performance liquid chromatography (HPLC) standards, including authentic flavonoids and phenolic acids, were provided by the Food Technology Research Institute under the Agriculture Research Center in Giza, Egypt.

2.3. Preparation of Crude Extracts

The leaves of *C. verum* J. Presl and *C. camphora* L. were dried at ambient temperature and separately pulverized into a fine powder (200 g per species). Each powdered sample underwent three successive extractions with 70% ethanol (3 × 500 mL) at room temperature. The pooled extracts were then evaporated under vacuum using a rotary evaporator (IKA RV 10 basic). The obtained crude extracts were stored at 25°C until further phytochemical evaluation.

2.4. Total phenolic acid content of the leaves of *C. verum* J. Presl and *C. camphora* L. by colorimetric method.

A Shimadzu UV-1601 PC spectrophotometer was used to assess the total phenolic content via the Folin–Ciocalteu method. The diluted extract (1 mL) was mixed with 5 mL of distilled water in a 10 mL flask for the assay. After thorough mixing with 0.5 mL Folin-Ciocalteu reagent, 1.5 mL of 5 g/L sodium carbonate solution was added following a 3-minute interval. The volume was then adjusted to 10 mL with distilled water, and the solution was incubated at 50°C for 16 minutes in sealed flasks using a water bath before being cooled to ambient temperature. Absorbance readings were taken at 765 nm against a distilled water blank. A gallic acid calibration curve (0–100 mg/L) was used as a reference, and results were reported as gallic acid equivalents (GAE) per

Vol. (2), No. (2), April-July; 2025. DOI:10.21608/MERMJ.2025.402345.1028

gram of fresh sample. To ensure reliability, all measurements were conducted in triplicate[5].

2.5. Determination of total flavonoids content of the leaves of *C. verum* J. Presl and *C. camphora* L. by colorimetric method Spectral analysis

An aluminum chloride colorimetric method was employed for flavonoid quantification. The sample extract (1 mL) was combined with 4 mL distilled water in a 10 mL flask. Sodium nitrite solution (0.3 mL, 0.5 g/L) was first added, followed after 5 minutes by aluminum chloride solution (0.3 mL, 1 g/L). After 6 additional minutes, the reaction was terminated with 2 mL of 1 mol/L sodium hydroxide. The final volume was adjusted to 10 mL with distilled water, and the mixture was vortexed before measuring absorbance at 510 nm using water as the blank. A rutin standard curve was prepared for quantification, with results expressed as rutin equivalents (mg RE/g) of the sample[6].

2.6. Determination of flavonoid compounds using HPLC analysis

For HPLC analysis, 5 g of desiccated leaf material was extracted with 40 mL of 62.5% methanol-water solution. The mixture underwent centrifugation at 1000 rpm for 10 minutes, after which the supernatant was passed through a 0.2 µm Millipore filter. The filtered extract was diluted to a final volume of 100 mL with methanol, and a 1:3 mL aliquot was transferred to an HPLC vial for injection, following the methodology described in reference [7]. Chromatographic identification of compounds was accomplished by matching sample retention times with those of authentic standards run under the same HPLC conditions. Quantitative analysis employed an external standard calibration approach, with compound concentrations determined through peak area integration. For flavonoid analysis, all determinations were carried out in triplicate, and the concentrations of the identified flavonoids were shown in **Table 1**.

2.7. Determination of phenolic compounds using HPLC analysis

[8] reported the method used for determination of phenolic acids by HPLC. The air-dried leaves (5 g) were mixed with 62.5% aqueous methanol (40 ml) and centrifuged at 1000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter and the filtrate was made up to 100 ml with methanol then 1:3 ml was collected in a vial for injection into the HPLC system. Identification of the individual components was performed as mentioned before in flavonoids. The concentration of the identified phenolic acids was shown in **Error! Reference source not found.**.

3. Results and Discussion

3.1 Total flavonoid and Total phenolic acids contents

Standard Preparation and Calibration: A series of dilutions were prepared from methanolic stock solutions (0.1 g/100 mL) of rutin (for flavonoid analysis) and gallic acid (for phenolic content determination). Absorbance measurements were recorded at 725 nm for each concentration in triplicate. Mean absorbance values were plotted against concentration to generate calibration curves. Quantitative results were reported as mean ± standard deviation, with flavonoids expressed in rutin equivalents (mg RE/g) and phenolic compounds as gallic acid equivalents (mg GAE/g).

Vol. (2), No. (2), April-July; 2025. DOI:10.21608/MERMJ.2025.402345.1028

Results	***C.verum***	***C.camphora***
Total Phenolics	20.61 mg GAE /g	19.65 mg GAE /g
Total Flavonoids	2.01 mg RE/g	3.88 mg RE/g

Table 1: Quantitative determination of total flavonoids and phenolic contents of the leaves of *C. verum* J.Presl and *C. camphora* L. by colorimetric assay.

Quantitative Analysis Results: Phenolic content analysis revealed absorbance values of 0.320 and 0.305 for C. verum and C. camphora leaf extracts, corresponding to 20.61 and 19.65 mg GAE/g dry weight, respectively.

Flavonoid quantification showed absorbances of 0.013 and 0.026 for the respective species, equivalent to 2.008 and 3.883 mg RE/g dry leaf material. As shown in Error! Reference source n ot found.**3.**

Flavonoids		***C.verum*** **mg/100g**	**(RT)**	**RRT**	***C.camphora*** ***mg/100g***	**(RT)**	**RRT**
1	**Naringin**	**44.34**	**12.876**	**0.802**	**116.6**	**12.735**	**0.911**
2	**Rutin**	**-**	**-**	**-**	**541.11**	**12.97**	**0.927**
3	**Hispertin**	**27.62**	**16.168**	**1.007**	**76.26**	**16.257**	**1.162**
4	**Hisperdin**	**13.19**	**13.019**	**0.811**	**156.68**	**13.057**	**0.934**
5	**Quercetrin**	**-**	**-**	**-**	**579.61**	**13.986**	**1.000**
6	**Quercetin**	**78.78**	**15.537**	**0.968**	**36.1**	**15.617**	**1.117**
7	**Narengenin**	**73.78**	**15.947**	**0.993**	**102.34**	**15.916**	**1.138**
8	**Kaempferol**	**97.83**	**16.056**	**1.000**	**98.08**	**16.147**	**1.155**
9	**Apegenin**	**37.82**	**17.206**	**1.072**	**115.93**	**17.311**	**1.238**
10	**7-OH flavone**	**160.32**	**18.14**	**1.130**	**114.7**	**18.13**	**1.296**

Table 2: Results of determination of flavonoids in the leaves of *C. verum* J. Presl and *C. camphora* L. using HPLC analysis.

Vol. (2), No. (2), April-July; 2025.

3.2. Qualitative and Quantitative determination of flavonoids and phenolic compounds in the leaves of *C. verum* J. Presl and *C. camphora* L. using HPLC analysis.

Flavonoids: Bioactive Polyphenolic Compounds

Flavonoids represent a diverse class of polyphenolic secondary metabolites ubiquitously present in medicinal plants, fruits, vegetables, and plant-derived beverages. Among these, quercetin derivatives have garnered significant research interest in recent years due to their potential health benefits as dietary components. Numerous studies have demonstrated the therapeutic potential of phenolic compounds against various pathological conditions, including atherosclerosis, neoplastic diseases, and inflammatory disorders [9].

Phenolic acids		*C.verum* mg/100g	(RT)	RRT	*C. camphora* mg/100g	(RT)	RRT
1	Gallic	0.49	7.079	0.531	4.62	7.107	0.508
2	Pyrogallol	1.93	7.2	0.540	6.19	7.158	0.512
3	4-Amino-benzoic	2.16	7.253	0.544	5.3	7.31	0.523
4	3-OH-Tyrosol	4.4	8.38	0.629	33.87	8.288	0.593
5	Protocatchuic	16.76	8.647	0.649	31.12	8.613	0.616
6	Catechein	5.24	8.76	0.657	13.89	8.763	0.627
7	Chlorogenic	11.51	9.4	0.705	150.74	9.41	0.673
8	Catechol	14.11	9.612	0.721	33.69	9.539	0.682
9	Caffeine	2.7	9.982	0.749	11.79	10.039	0.718
10	P-OH-benzoic	16.75	10.188	0.764	96.21	10.165	0.727
11	Caffeic	10.46	10.477	0.786	19.78	10.544	0.754
12	Vanillic	11.39	10.633	0.798	62.97	10.652	0.762
13	Ferulic	33.63	12.351	0.926	18.52	12.289	0.879
14	Iso-ferulic	26.74	12.664	0.950	65.36	12.673	0.906
15	Ellagic	27.62	13.688	1.027	74.86	13.624	0.974
16	e-vanillic	100.18	13.808	1.036	21.58	13.725	0.981
17	Benzoic	32.74	14.04	1.053	38.76	14.051	1.005
18	3,4,5-methoxy-cinnamic	18.23	14.637	1.098	53.4	14.584	1.043
19	Coumarin	37.29	14.713	1.104	131.33	14.713	1.052

Vol. (2), No. (2), April-July; 2025.

20	**Salycilic**	**76.06**	**14.949**	**1.121**	**177.9**	**15.107**	**1.080**
21	**p-coumaric**	**184.74**	**15.471**	**1.160**	**115.04**	**15.466**	**1.106**

Table 3: Results of determination of phenolic acids in the leaves of *C.verum* J.Presl and *C.camphora* L. using HPLC analysis**..**

Chromatographic Profiling of Leaf Extracts
High-performance liquid chromatographic analysis of *C. verum* J. Presl and *C. camphora* L. leaf extracts enabled the identification and quantification of 36 distinct phenolic constituents, comprising 10 flavonoid derivatives and 26 phenolic acid compounds.

7-OH, flavone and Kaempferol were the major flavonoids identified in the leaves of *C. verum* J. Presl with concentrations 160.32 and 97.83 mg/100g, respectively. While Quercetrin (579.61 mg/100g) and Rutin (541.11 mg/100g) were the major flavonoids observed in the leaves of *C. camphora* L.. The HPLC analysis of the phenolic acids of the leaves of *C. verum* J. Presl revealed the identification of Resveratrol (244.17 mg/100g) and p-coumaric (184.74 mg/100g) as major phenolic acids. Alpha-coumaric and Chlorogenic (257.36 and 150.74 mg/100g), respectively, were the major phenolic acids identified in the leaves of *C. camphora* L.

4. Conclusion

This study reveals that both *Cinnamomum* species *C. verum* J. Presl and *C. camphora* L. possess valuable phytochemical profiles, particularly in terms of phenolic and flavonoid content. The promising results from qualitative and quantitative analyses highlight the potential for future research focusing on compound isolation and purification from these plant extracts.

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Vol. (2), No. (2), April-July; 2025.

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