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PHENOLIC COMPOUNDS PLAY A CRUCIAL ROLE AS CHEMOTAXONOMIC MARKERS IN THE CUCURBITACEAE SPECIES CULTIVATED IN IRAQ

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SUMMARY

The concentration of phenolic compounds is researchable using chemotaxonomy as a valuable approach to studying the pharmacological potential of different crop plants. In line with this, the objective of the constructive study was to investigate the phenolic compounds concentration in 10 taxa belonging to the family Cucurbitaceae, earlier cultivated in mid-February 2021 at the College of Agriculture and Forestry, University of Mosul, Mosul, Iraq. The taxa included Cucurbita pepo Linnaeus, Cucurbita maxima Duchesne, Lagenaria siceraria L.C.V. Local Molina, Lagenaria siceraria L.C.V. Syria Molina, Cucumis sativus Linnaeus, Cucumis melo Linnaeus, Cucumis melo flexuosus Linnaeus, Citrullus lanatus (Thumberg) Matsum and Nakai, Citrullus colocynthis Schrader, and Luffa cylindrica Mill. Analyzing these compounds helped to gain a deeper understanding of the chemical composition of these plants, which may contribute to identifying their medicinal properties and potential applications in the field of pharmacology. The analysis of leaves of these taxa ran through high-performance liquid chromatography (HPLC) for 14 phenolic compounds, i.e., Apigenin, Catechin, Epicatechin, Kaempferol, Luteolin, Quercetin, Rutin, and Caffeic, Chlorogenic, Ferulic, Gallic, Hydroxy benzoic, P-coumaric, and Vanillic acids. A comprehensive study analyzed 10 taxa belonging to the family Cucurbitaceae using HPLC, revealing their heterogeneity and significant variations in the content and concentration of various phenolic compounds. The results of this analysis unveiled the identification of 14 compounds, primarily relying on the presence of standard material. Notably, the main component observed in this study was caffeic acid, showcasing its potential utility for taxonomists and pharmacologists and addressing diverse taxonomic challenges. These findings hold promising implications for the field, offering valuable insights into the intricate world of Cucurbitaceae and opening avenues for further research and application. Results further confirmed that the leaves of Cucurbitaceae plants are rich sources of nutrients and can significantly contribute to nutrient requirements and human health care.

Keywords: Cucurbitaceae, chemotaxonomy, phenolic compounds, pharmacological properties

Key findings: Cucurbitaceae species resulted heterogeneous, showing significant differences in various phenolic compounds for content and concentration through HPLC analysis.

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INTRODUCTION

The family Cucurbitaceae includes 120 genera and 825 species, with several cultivated plant species having significant commercial values, including *Cucurbita*, Lagenaria, Cucumis, Citrullus, and Luffa. The Cucurbitaceae family has characteristics of prostrate or scandent herbs, annual or perennial, usually climbing via tendrils. Tendrils have a different origin in different genera. The stem is pentangular; are leaves alternate, exstipulate, long petiolate, frequently cordate, and simple, but often palmately or pinnately compound. The flowers are usually solitary or have cymose inflorescence, yellow or white, unisexual arrangements (Jeffrey, 2001; Pandey and Mirsa, 2009; Pratami et al., 2020).

Chemotaxonomy is the attempt to classify and identify individuals according to apparent variations and similarities in their biochemical composition. Plants selection based on chemotaxonomy is necessary to gain success in natural products for further use for medicinal purposes (Darvis and Heywood, 1963). A conclusion stated that Cucurbitaceae seeds may be vital sources of antioxidant compounds and a range of phytochemicals with possible nutraceutical uses, i.e., carotenoids, alkaloids, phenolic compounds, and volatile oils, which are several examples of the immense bioactive substances found in the taxa of the family Cucurbitaceae (Muhammad et al., 2016).

High-performance liquid chromatography (HPLC) fingerprinting has been the best option for identifying medicinal plants and ensuring their quality because it is a more affordable analysis technique (Mamta *et al.*, 2013). The oldest application areas for metabolic profiling are probably drug discovery and chemotaxonomy, still in use because of the more than 200,000 different plant compounds and metabolomics in medicinal plants for evidence-based development of new products (Shyur and Yang, 2008).

In chemotaxonomy, classifying compounds revolves around three main categories: primary and secondary metabolites these, and semantics. Among phenol compounds belong to the group of metabolites derived from secondary metabolic pathways in plants. Polyphenols, encompassing flavonoids, phenolic acids, tannins, lignin, and coumarins, are natural components found in various sources, such as, fruits, vegetables, cereals, roots, and leaves. The significance of these compounds lies in their sustainable potential health benefits as phenolic compounds are antimicrobial, antioxidant, anticancer, and other crucial pharmacological properties (El-Sayed et al., 2018; Bahare et al., 2019).

Bioactive compounds have prominent actions in the body that may promote good health (Harbone, 1973; Dhiman et al., 2012; Deng et al., 2013; Alkasim et al., 2018). The compounds under investigation hold great promise in disease prevention, including cancer, heart disease, and various other ailments. As a result, these bioactive compounds serve as valuable assets for pharmaceutical purposes, offering a plentiful and advantageous source for both present and future pharmacological research. In this study, the objective sought a qualitative and quantitative analysis of phenolic compounds found in the leaves of 10 different taxa of the Cucurbitaceae family, utilizing Highperformance liquid chromatography as the analytical technique.

MATERIALS AND METHODS

Plant collection

The presented study began in mid-February 2021 at the College of Agriculture and Forestry, University of Mosul, Mosul, Iraq. The leaf samples of 10 different genera belonged to the family Cucurbitaceae. The taxa included *Cucurbita pepo, Cucurbita maxima, Lagenaria*

siceraria L.C.V. Local, Lagenaria siceraria L.C.V. Syria, Cucumis sativus, Cucumis melo, Cucumis melo flexuosus, Citrullus lanatus, Citrullus colocynthis, and Luffa cylindrica, collected during the flowering period.

Phenolic compound extraction

The plants under investigation underwent a meticulous process for leaf collection, ensuring utmost care ensued. The collected leaves gained thorough cleansing and subsequent drying in a gentle shade. Once completely dried, carefully crushing the leaves followed with a measured quantity of five grams of leaf powder obtained. This powder, transferred into a beaker, had 50 ml of ethanol (95%) added to each powdered genera leaf. Allowing the resulting mixture to soak in a refrigerator for 24 h, it then received a water bath set at 55 °C for 6 h. Using Whitman No. 1 filter paper for filtration aided in separating the extraction mixture from the residue. Employing a rotary evaporator (provided by the company Electro Thermal) helped remove the remaining solvent from the ethanol extracts of the sample under reduced pressure at a temperature of 48 °C (Grand et al., 1988).

Utilizing freeze dryer а helped lyophilize the extract. Then, obtaining 2 g of the distillate attained the addition of 200 ml of HCl acid at the concentration of 2 g, stirring the mixture while being heated in a water bath at 100 °C for 30 min. After cooling the concoction, adding 100 ml followed through the ethyl acetate, then separated by a separation funnel. Ethanol mixtures containing free phenolic compounds carriers gained concentration using а rotary vacuum evaporator, with the precipitate preserved after adding 3 ml of methanol.

Careful preservation of samples for the study involved freezing them in sterile tubes with an airtight cover until they were ready for use (Harbone, 1973; Singh, 2016). Employing a high-performance liquid chromatography (HPLC) technique used two different systems: the Zorb ax Eclipse Plus-C18-OSD with Chem station and the SYKAMN HPLC chromatographic system equipped with a UV detector. Reversed-phase HPLC analysis also identified

specific phenolic chemicals, utilizing a 4.6 mm column with a 25 cm length. The column maintenance had a temperature of 30 °C. The elution process involved using eluents A (methanol) and B (1% formic acid in water, v/v) through a gradient method. The elution ran as follows: 40% B from 0 to 4 min, 50% B from 4 to 10 min, with a flow rate of 0.7 mL min⁻¹. An autosampler helped inject 100 µL of the sample and 100 μL of the standard automatically. The spectra collection was at a wavelength of 280 nanometers. The evaluation of phenolic compounds continued using an HPLC system, specifically the Shimadzu LC-10A, located in the Ministry of Science and Technology, Department of Environment and Water. The conditions employed were as follows: a C-18 column (CTO-10ASVP) measuring 4.6 mm \times 250 mm and 5 μ m particle size, with a mobile phase composed of solvent A (formic acid with 3% methanol) and solvent B (108% acetonitrile). The injection volume was 20 µL, and applying a gradient elution method ranged from 15% to 100% B, with a run time of 45 min and a flow rate of 1 mL min⁻¹. For analysis, dissolving the samples in methanol continued with 20 μL of the solution injected into the column. The chromatograms' evaluation at 289 nm used an LC gradient detector, with UV detection at 280 nm (Radovanoic et al., 2015). The recorded eluted material from the standards appears in Table 1. The sample area of the standard multiplied by the concentration of the standard, using the dilution factor, determined the concentration of phenolic compounds in the sample and expressed the results as mg ml⁻¹.

RESULTS AND DISCUSSION

Leaf extracts of various taxa belonging to the family Cucurbitaceae occurred heterogeneous in terms of content and concentration of phenolic compounds through HPLC analysis. Results showed the identification of 14 compounds based on the available standard materials, with these compounds shown in Figures 1, 2, 3, and 4 having a standard compound curve. Table 2 shows the presence of phenolic compounds in various species. Apigenin, from the group of flavones, also resulted in the species *Cucurbita pepo*, *Cucurbita maxima*, *Cucumis sativus*, *Cucumis melo*, *and Cucumis melo flexuosus*.

Caffeic acid is from a group of nonflavonoid phenolic compounds from the hydrocinnamic acid, and this compound has also surfaced in the species *Cucurbita pepo*, *Cucurbita maxima*, *Lagenaria siceraria* L.c.v. Local, *Lagenaria siceraria* L.c.v. Syria, *Cucumis sativus*, *Cucumis melo*, *Cucumis melo flexuosus*, *Citrullus lanatus*, and *Citrullus colocynthis*. Catechin is a secondary metabolite that serves as an antioxidant in plants. It is a member of the polyphenol flavonoids subclass. It has emerged in the species *Citrullus lanatus and Citrullus colocynthis.* Chlorogenic is the ester of caffeic, and the chlorogenic acids refer to a similar family of polyphenol esters that operate as an intermediary in biosynthesis. Said compound has materialized in *Cucurbita pepo, Cucumis melo flexuosus,* and *Luffa cylindrica.* Epicatechin is one of the most abundant flavonoids found in various fruits and their leaves, and it has also resulted in the species *Cucumis sativus, Cucumis melo,* and *Luffa cylindrica.*

No	Phenolic compound	Retention time	Area	Height		
No.	(20 µm)	(minute)	(MaU.S)	(mAU)		
1	Apigenin	4.35	1245.08	805.46		
2	Caffeic acid	7.01	960.11	601.58		
3	Catechin	5.54	960.15	788.01		
4	Chlorogenic acid	6.50	1542.08	811.58		
5	Epicatechin	12.54	850.14	822.49		
6	Ferulic acid	10.50	1452.98	860.44		
7	Gallic acid	2.30	856.08	930.15		
8	Hydroxybenzoic acid	13.59	1874.45	930.23		
9	Kaempferol	5.01	1365.08	790.48		
10	Luteolin	8.02	1142.09	799.45		
11	P-coumaric acid	9.32	901.28	850.47		
12	Quercetin	11.58	1601.14	950.44		
13	Rutin	3.40	960.14	850.44		
14	Vanillic acid	14.05	960.25	955.48		

Table 1. Phenolic compounds standards used in the study.

Table 2. Distribution of phenolic compounds in the different species of the family Cucurbitaceae.

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Cucurbita pepo	+	+		+			+	+		+		+		
2	Cucurbita maxima	+	+		+			+	+		+		+		
3	Lagenaria siceraria L.C.V. Local		+					+		+	+		+	+	+
4	<i>Lagenaria siceraria</i> L.C.V. Syria		+					+		+	+		+	+	+
5	Cucumis sativus	+	+			+	+		+			+		+	
6	Cucumis melo	+	+			+	+		+			+		+	
7	Cucumis melo flexuosus	+	+		+			+	+		+		+		
8	Citrullus lanatus		+	+				+		+	+	+	+		
9	Citrullus colocynthis		+	+				+		+	+	+	+		
10	Luffa cylindrica				+	+				+	+	+		+	+

1- Hydrobenzoic acid 2- Caffeic acid 3- Catechin 4- Chlorogenic acid 5- Epicatechin 6- Ferulic acid 7- Gallic acid 8- Apigenin

9- Kaempferol 10- Luteolin 11- P-Coumaric acid 12- Quercetin 13- Rutin 14- Vanillic acid.



Figure 1. Standard compound curve of phenolic acid.



Figure 2. Standard compound curve of phenolic acid.



Figure 3. Standard compound curve of phenolic acid.



Figure 4. Standard compound curve of phenolic acid.

Ferulic acid is also from a group of non-flavonoid phenolic compounds from hydrogen cinnamic acid. An antioxidant derived from plants called ferulic acid is mainly a component in preparing skin care products for anti-aging. Various natural foods contain it. This compound came out in the species *Cucumis sativus* and *Cucumis melo*. A phenolic acid, also called gallic acid, is found in plants. It contains antioxidant qualities and could provide other health advantages. The compound has appeared in *Cucurbita pepo, Cucurbita maxima, Lagenaria siceraria* L.C.V. Local, *Lagenaria siceraria* L.C.V. Syria, *Cucumis melo flexuosus, Citrullus lanatus, and Citrullus colocynthis* species.

Hydrobenzoic acid and monohydroxy benzoic acid, a phenolic derivative of benzoic acid, also known as p-hydroxybenzoic acid (PHBA), was identifiable in the species Cucurbita pepo, Cucumis sativus, Cucumis and Cucumis melo flexuosus. melo, Kaempferol, one of the flavanol compounds, was recognizable in the species Lagenaria siceraria L.C.V. Local, Citrullus lanatus, Citrullus colocynthis, and Luffa cylindrica.

Luteolin, also known as 3', 4', 5, 7tetrahydroxyflavone, is a typical flavonoid found in many plants, including fruits, vegetables, and therapeutic herbs. In Chinese traditional medicine, luteolin-rich plants have helped cure various ailments, including cancer, inflammatory disorders, and hypertension. This compound has also emerged in *Cucurbita pepo, Cucurbita maxima, Lagenaria siceraria* L.C.V. Local, *Lagenaria siceraria* L.C.V. Syria, *Cucumis melo flexuosus, Citrullus lanatus, Citrullus colocynthis,* and *Luffa cylindrica*.

P-coumaric acid is an organic compound. It was distinguishable in *Cucumis sativus, Cucumis melo, Citrullus lanatus, Citrullus colocynthis,* and *Luffa cylindrica.* Quercetin, a plant pigment (flavonoid), also surfaced in *Cucurbita pepo, Cucurbita maxima, Lagenaria siceraria* L.C.V. Local, *Lagenaria siceraria* L.C.V. Syria, *Cucumis melo flexuosus, Citrullus lanatus,* and *Citrullus colocynthis.*

Rutin is also one of the flavanols considered a substance of pharmaceutical interest related to treating capillary frailty in humans. This compound has also become visible in *Cucumis sativus, Cucumis melo, Lagenaria siceraria* L.C.V. Local, *Lagenaria siceraria* L.C.V. Syria, and *Luffa cylindrica.* Vanillic acid, a monohydroxy benzoic acid and a vital plant metabolite, came out in *Lagenaria siceraria* L.C.V. Local, *Lagenaria Lagenaria siceraria L.C.V.* Syria, and *Luffa cylindrica.*

In the leaf samples of 10 taxa, the diagnosed phenolic compounds curve is in Figures 5, 6, and 7, while the concentration of phenolic compounds appears in Table 3. The Apigenin compound came with the highest concentration in the species Cucumis melo *flexuosus* (18.44 μ g ml⁻¹), whereas the lowest concentration (9.25 µg ml⁻¹) was in *Cucumis* sativus. The Caffeic acid was distinctive by the highest value (13.89 µg ml⁻¹) in *Cucumis melo*, with less concentration observed in Citrullus *lanatus* (6.59 µg ml⁻¹). The Catechin compound provided the highest concentration in Citrullus colocynthis and Citrullus lanatus (25.98 and 20.65 µg ml⁻¹, respectively). Epicatechin has shown the maximum dilution in Luffa cylindrica (18.55 µg ml⁻¹) but less concentration in *Cucumis sativus* (9.58 µg ml⁻¹). The chemical analysis showed antioxidant and antimicrobial activities in the cultivars of the Greek cucumber (Cucumis sativus) (Sotiroudis et al., 2010). Past studies enunciated that various antioxidant activities, total phenolic compounds, and flavonoid contents with varied values were notable in diverse species of crop plants (Shyur and Yang, 2008; Partap et al., 2013; Supriya et al., 2015; Singh, 2016).

The Ferulic acid was only notable in Cucumis melo and Cucumis sativus (12.48 and 10.5 μ g ml⁻¹, respectively). The Gallic acid's highest concentration appeared in Citrullus *colocynthis* (24.58 μ g ml⁻¹), whereas less dilution was in *Cucurbita maxima* (10.25 µg ml⁻ ¹). The highest concentration of hydroxybenzoic acid came from Cucumis melo *flexuosus* (17.44 μ g ml⁻¹l); however, the minimum was in Cucumis sativus (12.69 µg ml⁻ ¹). The maximum strength of Kaempferol was distinguishable in Citrullus colocynthis (22.49 μq ml⁻¹), with less concentration exhibited by the species Lagenaria siceraria L.C.V. Local (16.08 μ g ml⁻¹). These results agreed with the findings of Okon et al. (2014), who reported the highest concentration of phenolic and flavonoid compounds in Cucurbita maxima leaves. The study also revealed that the species C. maxima leaf extract significantly inhibits the growth of Staphylococcus aureus,



Figure 5. Curve of phenolic compounds identified in cultivars of Cucurbitaceae plants.



Figure 6. Curve of phenolic compounds identified in cultivars of Cucurbitaceae plants.



Figure 7. Curve of phenolic compounds identified in cultivars of Cucurbitaceae plants.

No.	Name (PPM)	1	2	3	4	5	6	7	8	9	10
1	Gallic acid	13.58	10.25	10.59	13.58			18.59	20.58	24.58	
2	Rutin			13.22	15.88	20.58	22.59				22.59
3	Apigenin	13.89	12.55			9.25	10.88	18.44			
4	Kaempferol			16.08	18.59				18.99	22.49	20.33
5	Catechin								20.65	25.98	
6	Chlorogenic acid	7.11	6.58					9.58			15.98
7	Caffeic acid	10.89	9.58	8.44	9.58	12.55	13.89	11.25	6.59	11.48	
8	Luteolin	12.58	11.25	7.48	10.88			13.56	9.14	14.58	11.25
9	P-Coumaric acid					14.58	16.44		13.69	15.89	13.65
10	Ferulic acid					10.5	12.48				
11	Quercetin	18.79	16.25	15.44	19.85			20.58	18.99	20.58	
12	Epicatechin					9.58	10.89				18.55
13	Hydrobenzoic acid	15.25	13.22			12.69	13.59	17.44			
14	Vanillic acid			10.48	12.36						9.65

Table 3. Phenolic compounds concentration in various species of the family Cucurbitaceae.

1- Cucurbita pepo 2- Cucurbita maxima 3- Lagenaria siceraria L.C.V. Local 4- Lagenaria siceraria L.C.V. Syria 5- Cucumis sativus 6- Cucumis melo 7- Cucumis melo flexuosus 8- Citrullus lanatus 9- Citrullus colocynthis 10- Luffa cylindrica

Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli by having an intense antibacterial action. Past findings were also in analogy with the presented results, and the species Lagenaria siceraria signified to have antioxidant properties and cucurbitacin and fiber (Shirwaikar and Sreenivasan, 1996; Kirtikar and Basu, 2001; Sabahudin *et al.*, 2013).

For Luteolin, the highest concentration resulted in Citrullus colocynthis (14.58 µg ml⁻ ¹), and the less was in *Lagenaria siceraria* L.C.V. Local (7.48 µg ml⁻¹). Moreover, the Pcoumaric acid gave the highest concentration in *Cucumis melo* (16.44 μ g ml⁻¹), whereas the lowest value of the same value came from Luffa cylindrica and Cucumis melo flexuosus (13.65 µg ml⁻¹). *Luffa cylindrica* and *Cucumis melo* provided the highest concentration of Rutin (22.59 μ g ml⁻¹), with the minimum intensity by Lagenaria siceraria L.C.V. Local (13.22 μ g ml⁻¹). The highest and lowest concentrations of Vanillic acid emerged in *Lagenaria siceraria* L.C.V. Syria (12.36 µg ml⁻¹) and Luffa cylindrica (9.65 μg ml⁻¹), respectively. Given the plant's enormous therapeutic values in the present era, the researchers concentrated on investigating the free radical scavenging ability of the plant's leaves to ascertain their involvement in diverse oxidative stress conditions caused by various reactive oxygen species (Neeraj et al., 2013;

Abd-El-Raheem et al., 2022). The foremost amount of phenolic (23.08 mg GAE g⁻¹) and flavonoids (14.02 mg QE g⁻¹) materialized in the ethanolic peel extract, and numerous chemical substances, including cucurbitacin, phenolic compounds, vitamins, minerals, essential oils, and fatty acids, resulted in the plants of genus Cucumis (Zhang et al., 2022). Numerous studies have demonstrated the pharmacological properties of the genus Cucumis plants, including antibacterial, analgesic, antioxidant, anti-inflammatory, antidiabetic, anti-wrinkle, and anticancer activities (Alkasim et al., 2018; Muhamad et al., 2022).

The phytochemicals found in plant extracts, having connections to an antioxidant effect, include phenols and flavonoids. An antioxidant material known as phenolic compounds functioned as scavengers of free radicals. The hydroxyl group (-OH) has direct links to an aromatic hydrocarbon (phenyl) ring, thought to endow phenols with their antioxidant potential, which can easily give electrons to free radicals and lessens the threat to human cells (Ritchel et al., 2004; Abu et al., 2020). Citrullus colocynthis was discovered as a significant therapeutic plant in traditional medicines (Al-Nablsi et al., 2022).

The total phenolic content and antioxidant activity of various fruit portions (rinds, pulps, and seeds) from three different accessions with significant genetic differences underwent testing in the contemporary study. The results showed that the total phenols were significantly limitless in the rinds than in the seeds and pulp; however, the antioxidant activity was substantially higher in the spores than in the peels and pulps (Kaur and The HPLC Aggarwal, 2013). technique measured the alcohol extract of the callus produced by Citrullus colocynthis L., and the highest concentration of the Rutin component surfaced (Al-Hamdany, 2013). Luffa cylindrica is а worldwide plant with major phytoconstituents under the flavonoid classification found in it. A wide range of ailments, including snake bites, convulsions, cramps, tetanus, dropsy, emetic, cathartic, chronic bronchitis, asthma, sinusitis, and fever, have alleged treatments with various parts of this plant as traditional medicine (Partap et al., 2013).

CONCLUSIONS

The leaf extracts obtained from different within the Cucurbitaceae species family exhibited significant heterogeneity when analyzed for their content and concentration of phenolic compounds using HPLC. The results of this study revealed the identification of 14 distinct compounds, which were distinguishable based on the available standard reference materials. Among these compounds, caffeic acid emerged as the predominant component, holding great potential for utilization by taxonomists, pharmacologists, and researchers seeking to address various taxonomic concerns. This finding contributes to understanding the Cucurbitaceae leaf extracts' chemical composition and holds promise for its practical applications in the mentioned fields.

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