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Genetic and Chemical Diversity of *Cuminum cyminum* L. and *Bunium bulbocastanum* L.: A Comparative Study Using RAPD, SCoT Molecular Markers and Phytochemical Analysis

Shayma'a Mazee Jabbar[1]*, Taif Al-saadi*[2], Attyaf Jameel Thamir ALTamimi[3] and Nidhal Abdul Hussein Al-Bdairi[4]

[1,3,4,] *University of Kufa, College of Science, Iraq.*

[2,] *Al-Furat Al-Awsat Technical University, Polytechnic College, Kufa, Iraq.*

***Corresponding Author Email:** shaimaam.hassoun@uokufa.edu.iq, taif.najjar@atu.edu.iq, atyaf.altameemi@uokufa.edu.iq, nidhal.albdairi@uokufa.edu.iq

ABSTRACT

Background: *Cuminum cyminum L. (Cumn) and Bunium bulbocastanum L. (Buni), are members of the Apiaceae family, which contains economically and medicinally important aromatic plants commonly used in traditional medicine and culinary practices on a large scale in Iraq and the Middle East regions. The aim of this study was to investigate the genetic and chemical diversity between both species using RAPD & SCoT molecular markers as well as chemical analysis by GC-MS, HPLC quantification of flavonoids, and physicochemical characterization of essential oils. Genetic assessment was performed with the help of five RAPD and five SCoT primers. Hydrodistillation was performed to extract essential oils and HPLC analysis was conducted for identifying flavonoids. The results showed a high degree of genetic variation, and the SCoT markers displayed more polymorphic bands (49), than RAPD markers (19) representations. Gas chromatography--mass spectrometry analysis revealed that C. cyminum oil was dominated by cuminaldehyde (42.43%) whereas B. bulbocastanum oil was characterized by thymol (35.5%) and carvacrol (15.53%; 3). The total flavonoid content of the species was similar (607.44 and 603.87 µg/mL) with apigenin as the most abundant compound. B. bulbocastanum (2.125%) and C. cyminum (1.729%) released a relatively higher yield of the essential oils being of good quality in both cases, but the first plant outperformed the other one. The results scientifically validate traditional uses and pave the way for future industrial applications, particularly in pharmaceuticals, food production, cosmetics and provides a platform for conservation and selective breeding strategies*

Keywords: **HPLC, GC-MS, Flavonoids, Essential oil, SCoT, RAPD, Genetic diversity, Bunium bulbocastanum, Cuminum cyminum**.

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INTRODUCTION

The genus Cuminum belongs to family Apiaceae, which include aromatic plants with remarkable commercial and medicinal values, in which the cumin Cuminum cyminum L. is among the most widely cultivated and consumed spice crops worldwide (Shaaban Hamdy, 2023). Likewise, the other black cumin or earthnut *Bunium bulbocastanum L.*, also belongs in the same botanical family and is a folk medicine in several Asian and Middle Eastern folk medicine systems (Al-Mansour et al., 2024; Dehghani et al., 2024). The culinary and therapeutic uses of both species highlight their unique aromatic profiles and diverse reserves of bioactive secondary metabolites, such as essential oils and flavonoids. These plants are parts and parcels of traditional medicine and ethnic food preparation in Iraq and neighboring countries (Acimovic *et al.*, 2024).

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The assessment of genetic diversity is critical to the characterization and conservation and utilization of plant genetic resources. Molecular markers have been used efficiently for assessing genetic diversity as they are independent of environmental effects (Govindaraj et al., 2015). Random Amplified Polymorphic DNA (RAPD) and Start Codon Targeted (SCoT) markers have been applied extensively as molecular marker systems to study genetic diversity in medicinal and aromatic plants due to their simplicity, reproducibility and low-cost (Collard & Mackill, 2009). These markers have been widely used to make the genetic characterization of Apiaceae species. Nevertheless, there are still few studies regarding molecular characterization of *B. bulbocastanum* using these marker systems especially for Iraqi genotypes (Sofalian *et al*., 2008; Singh & Sengar, 2015).

Seeds of cumin (Cuminum cyminum) have a characteristic aroma and taste due mainly to their respective composition of essential oils. Some major volatile components of CMEO are cuminaldehyde, γ-Terpinene, p-cymene, β-Pinene and limonene, of which the major component is cuminaldehyde (Ouryemchi et al., 2024; Fidan et al., 2025). Nonetheless, an extensive study by Acimovic et al. (2024) showed that the main components of cumin essential oil were cumin aldehyde (32.13%), γ-terpinene (20.19%), and β-pinene (15.86%). An average of the essential oil content of cumin seeds from the above studies shows that it can be from 1.5% to 3.4% depending on agronomic practices (Shaaban Hamdy, 2023). Moreover, cumin essential oil has shown strong antifungal activity against phytopathogenic fungi (Msegued Ayam *et al*., 2025).

Black cumin (*Bunium bulbocastanum L.*) has received a lot of interest for its pharmacological value. In contrast with the widely studied *B. persicum, B. bulbocastanum* was only recently a notable subject for scientific study owing to its appealing chemical composition (Al-Mansour et al., 2024; Dehghani et al., 2024). The essential oil of *B. bulbocastanum* is well-known for high amounts of thymol, which is a natural phenolic monoterpene with a great antimicrobial and antioxidant activity (Ari et al., 2024; Yilmaz & Turan, 2025). In the most recent metabolomic profiling study of seeds of the Iraqi *B. bulbocastanum*, 35 metabolites were identified; with thymoquinone (5.24%) and carvacrol (4.78%) being among the most abundant (Hama & Omer, 2024). Other common species include apigenin, kaempferol, rutin, and quercetin, the antioxidant activities of which are well-documented term (Kashkolinia *et al*., 2024; Bendifallah *et al*., 2025).

Although both species are recognized as medicinally and commercially important, few studies characterize molecular genetic diversity alongside phytochemical profiling. Compared to *C. cyminum*, the cuisine show more limited attention in the scientific literature for *B. bulbocastanum* (Dehghani et al., 2024). Herein, we designed the current study to fill the knowledge gap through the following objectives: (1) evaluation of genetic diversity between C. cyminum and *B. bulbocastanum* using five RAPD and five SCoT primers, (2) extraction and quantification of essential oils by hydrodistillation, (3) identification of essential oil compounds using GC–MS, (4) quantification of flavonoids by HPLC, (5) assessment of physicochemical properties for both essential oils, and (6) comparative analysis of genetic and chemical profiles between both species. These findings will aid in scientifically validating medical applications and serve as a basis for all industrial applications in medicines, food and cosmetics.

MATERIALS AND METHODS

1. Plant Materials

Cuminum cyminum L. (local cumin) and *B. bulbocastanum L.* (black cumin) seeds were

purchased from local Iraq markets. The plant materials were identified and authenticated by a plant taxonomist at University of Kufa, Iraq. The seeds were washed with distilled water to remove dust and contaminants, were shade-dried at room temperature (25 ± 2°C) for 72 hours until constant weight. Dried seeds were powdered in an electric grinder and segregated into airtight bags at 4 °C until use (Shaaban Hamdy, 2023).

2. Molecular Genetic Analysis

2.1. Genomic DNA Extraction

Genomic DNA was obtained from 100 mg of young leaf tissue or seed powder of both *B. bulbocastanum and C. cyminum* using a modified CTAB (Cetyltrimethylammonium bromide) method according to Doyle and Doyle (1987). DNA purity was assessed using a spectrophotometer, with A260/A280 absorbance ratios ranging from 1.8 to 2.0. The measured concentrations were diluted to a standard working concentration of 50 ng/µL using nuclease-free water (Govindaraj *et al*., 2015).

2.2. RAPD-PCR Amplification

Five RAPD primers were used in this study: OPA-01, OPA-03, OPA-10, OPB-06, and OPD-13. The sequences and annealing temperatures of these primers are presented in Table 1 (Carelli *et al*., 2006; Abd El-Hady *et al*., 2010; Ezekiel *et al*., 2011; El-Assal & Gaber, 2012).

2.3. SCoT-PCR Amplification

Five SCoT primers were used: SCoT9, SCoT12, SCoT26, SCoT30, and SCoT44. The sequences and annealing temperatures of these primers are presented in Table 2 (Vivodik *et al*., 2017).

Table 1. RAPD primers used in the study.

No.	Primer Name	Sequence (5′→3′)	Annealing Temperature (°C)
1	OPA-10	GTGATCGCAG	40
2	OPA-03	AGTCAGCCAC	40
3	OPA-01	CAGGCCCTTC	40
4	OPB-06	TGCTCTGCCC	37
5	OPD-13	GGGGTGACGA	40

Table 2. SCoT primers used in the study.

No.	Primer Name	Sequence (5′→3′)	Annealing Temperature (°C)
1	SCoT 9	CAACAATGGCTACCAGCA	50
2	SCoT 30	CCATGGCTACCACCGGCG	50
3	SCoT 44	CAATGGCTACCATTAGCC	50
4	SCoT 26	ACCATGGCTACCACCGTC	50
5	SCoT 12	ACGACATGGCGACCAACG	50

2.4. PCR Amplification Conditions

RAPD-PCR Program: The PCR reaction mixture was prepared in a final volume of 25 µL containing 50 ng of template DNA, 1× PCR buffer, 0.2 mM of each dNTP, 0.4 µM of primer, 1 U of *Taq* DNA polymerase, and nuclease-free water. The amplification program consisted of an initial denaturation at 94°C for 3 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at variable temperatures (37–40°C) for 1 minute, and extension at 72°C for 1 minute, with a final extension step at 72°C for 5 minutes (Carelli *et al*., 2006; Abd El-Hady *et al*., 2010).

SCoT-PCR Program: The amplification program consisted of an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, and extension at 72°C for 2 minutes, with a final extension step at 72°C for 5 minutes (Vivodik *et al*., 2017; Collard & Mackill, 2009).

2.5. Gel Electrophoresis

The PCR products were separated by gel electrophoresis on a 1.5% agarose gel in 1× TAE buffer. The amplified fragments (10 µL per reaction) were separated by size at 80 V for approximately 2 hours and stained with ethidium bromide. The gels were visualized using a UV transilluminator and a gel documentation system. A 100-base-pair DNA ladder was used as a molecular size standard to estimate fragment lengths (Collard & Mackill, 2009).

2.6. Data Analysis

Band patterns were recorded as binary data (1 for presence of band, 0 for absence). Only clear, reproducible, and well-resolved bands were scored for analysis. Data were analyzed using NTSYS-pc (version 2.10) software to calculate the total number of bands, number of polymorphic bands, and percentage polymorphism (Govindaraj *et al*., 2015).

3. Essential Oil Extraction by Hydrodistillation

The essential oil was extracted from the powdered seed samples (50 g of each species) using a Clevenger-type apparatus according to the standard hydrodistillation method described by the European Pharmacopoeia (Zhang *et al*., 2018). The ground seed material was placed in a 500 mL round-bottom flask, and 500 mL of distilled water was added (1:10 ratio). The Clevenger apparatus was assembled by attaching the boiling flask to the hydrodistillation receiver and condenser. Anti-bumping granules were added to prevent violent boiling.

The mixture was boiled with the aid of a heating mantle, and hydrodistillation continued for 3–4 hours until no more essential oil was obtained (Samadi et al., 2020). The essential oil was separated from the aqueous phase (hydrosol) using a separatory funnel, dried over anhydrous sodium sulfate (Na_2SO_4) to remove residual water, and subsequently filtered. The pure oil was kept in amber color glass vials at 4°C till further analyses (Shaaban Hamdy, 2023).

Essential Oil Yield Calculation: The essential oil yield was measured as the percentage (v/w) according to the dry weight of the plant material using the following formula:

Oil yield (%) = (Volume of essential oil (mL) / Weight of dry plant material (g)) × 100

The extractions have been repeated in triplicate, and findings were presented as mean ± standard deviation (Acimovic *et al*., 2024).

4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

4.1. Sample Preparation

The essential oil (50 µL) has been dissolved in 1 mL of n-hexane. The mixture was vortexed for 30 seconds and filtered through a 0.45 µm syringe filter into a GC vial (Ouryemchi *et al*., 2024).

4.2. GC-MS Conditions

GC-MS analysis was performed using a Shimadzu GCMS-QP2010 Plus system (Shimadzu, Japan) equipped with an AOC-20i autosampler and a split/splitless injector. The separation was carried out on a fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.46 mL/min.

The GC oven temperature program was set as follows: initial temperature 80°C held for 2 minutes, then increased to 300°C at a rate of 10°C/min, and held at 300°C for 6 minutes. The injector temperature was maintained at 300°C, and the injection mode was split with a split ratio of 5:1. The injection volume was 1 µL (Acimovic *et al*., 2024; Fidan *et al*., 2025).

The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. The ion source temperature was set at 200°C, and the interface temperature was maintained at 250°C. The solvent cut time was 2.00 minutes. Mass spectra were acquired over the mass range of m/z 40–800 at a scan speed of 1666 (Ouryemchi *et al*., 2024).

4.3. Compound Identification

The identification of volatile compounds (cuminaldehyde, γ-terpinene, p-cymene, β-pinene, limonene, thymol, β-myrcene, caryophyllene, and carvacrol) was performed by comparing the mass spectra with the NIST20 (National Institute of Standards and Technology) mass spectral library. A compound was considered positively identified when the match factor (SI) exceeded 80% (Msegued Ayam *et al*., 2025; Yilmaz & Turan, 2025).

5. High-Performance Liquid Chromatography (HPLC) Analysis of Flavonoids

5.1. Sample Preparation for HPLC

One gram of dried seed powder was extracted with 20 mL of hexane to remove fat. The defatted residue was dissolved in 120 mL of methanol:water (80:20, v/v). The extract was subjected to ultrasonication (Branson sonifier, USA) at 60% duty cycles for 25 minutes at 25°C, followed by centrifugation at 7,500 rpm for 15 minutes. The clear supernatant was evaporated under vacuum using a rotary evaporator (Buchi Rotavapor). Dried samples were re-suspended in 1.0 mL of HPLC-grade methanol by vortexing, passed through a 2.5 µm disposable filter, and stored at 4°C for further analysis (Bendifallah *et al*., 2025).

5.2. HPLC Conditions

Separation of flavonoid compounds was performed on a Shimadzu 10AV-LC system equipped with a binary delivery pump (LC-10A Shimadzu) and a UV-Vis spectrophotometric detector (SPD-10A). A Phenomenex C-18 column (50 × 4.6 mm I.D.; 3 µm particle size) was employed. Temperature at column = 30°C.

The mobile phase: solvent A (water + 0,1% formic acid). solvent B (ACN + 0,1% formic acid). The flow rate was 1.2 mL/min. Detection was performed at 280 nm. Injection volume: 20 µL (Kashkolinia *et al*., 2024).

5.3. Compound Identification and Quantification

The identification of flavonoid compounds (apigenin, kaempferol, rutin, and quercetin) was carried out by comparing their retention times with authentic standards. The concentration of each compound was assessed by the calculation:

Therefore, the concentration of sample (µg/mL) = (Area of sample / Area of standard) × Concentration of standard × Dilution Factor

Results were reported as µg/mL or mg/g of dry weight (Bendifallah et al., 2025; Kashkolinia et al., 2024).

6. Physicochemical Properties of Essential Oils

6.1. Materials and Chemicals

Analytical grade reagents were used for the experimental study. Solutions were freshly

prepared before analysis. All oil samples were preserved in amber glass bottles at 4 °C until the time of analysis to prevent oxidative degradation (Shaaban Hamdy, 2023).

6.2. Sample Preparation

Oil extracts were filtered using Whatman No. 1 filter paper to get rid of debris. Samples were equilibrated at 25 ± 2°C and homogenized before analysis. All measurements were done in triplicate and results were expressed as mean ± standard deviation (Acimovic et al., 2024).

6.3. Moisture Content

Moisture content determination was carried out by the oven-drying method. An approximate weight of 5 g of oil sample was weighed into a crucible that was pre-dried and heated at a temperature of 105°C until constant weight. The moisture content was determined by the relative change in weight (percentage weight lost from the initial weight) (Shaaban Hamdy, 2023).

6.4. Density

The oil density was measured at 25 °C (at a very accurate temperature) using a pycnometer. The pycnometer is calibrated with distilled water before its use. The density was determined from the mass of the oil divided by the known volume of the pycnometer (Acimovic et al, 2024).

6.5. Refractive Index

At 25 °C, the refractive index was measured using an Abbe refractometer. A drop of oil is applied on the prism surface and readings were captured after reaching temperature stability (Shaaban Hamdy, 2023).

6.6. Optical Rotation

Optical rotation measurements were performed at 25°C using a polarimeter and the specific rotation calculated as described in standard methods (Acimovic et al., 2024).

Statistical Analysis

All experiments were conducted in triplicate, and the data was presented as mean ± SD. Microsoft Excel 2019 and SPSS (version 25) software were used for statistical analysis. Three-tailed significance was determined via $p < 0.05$ where applicable (Govindaraj *et al*., 2015; Shaaban Hamdy, 2023).

RESULTS

3.1. Genetic Diversity Using RAPD and SCoT Markers

3.1.1. Genetic Diversity Analysis Using RAPD Markers

A total of five RAPD (OPA-01, OPA-03, OPA-10, OPB-06, OPD-13) primers were used to determine the genetic variation of local cumin (C. cyminum) and black cumin (*B. bulbocastanum*) samples. The results showed distinct differences in banding patterns between the two species, which reported significant genetic diversity (Govindaraj et al., 2015). In total, 23 polymorphic bands were observed varies in the size from 100 to 1106 base pairs. A total of 99 bands were produced with all the selected primers (Table 2). Primer OPD-13 showed a maximum of 10 polymorphic bands, and primers OPA-10 and OPB-06 displayed well-differentiated polymorphism at 900, 783, 500, and 366 base pairs (Figure 1). Sample 2 (black cumin) showed two banding pattern at 300 base pair with primer OPD-13, and the band appeared at 283 base pair with primer OPA-01. This result suggests that RAPD markers have the potential to differentiate between these studied species, which is in accordance with many earlier studies that used RAPD in the assessment of genetic diversity in species belonging to the Apiaceae family (Sofalian *et al*., 2008; Singh & Sengar, 2015).

3.1.2. Genetic Diversity Analysis Using SCoT Markers

Genetic Diversity Assessment Using SCoT Primers SCoT9, SCoT12, SCoT26, SCoT30 and SCoT44 Banding patterns showed high polymorphism (band sizes from 290 to 2058 base pairs, shown in Figure 2) between both species Primer SCoT44 easily differentiated between the samples, exhibiting clear bands in sample 1 (local cumin) at 2058 and 1753 base

pairs, and a distinctive band at 1517 base pairs in sample 2. Primer SCoT30 presented a general polymorphism with the presence of polymorphic bands in 1450, 1134, 1043, 957, 886, 747 and 574 base pairs. The higher discrimination of SCoT markers by far than RAPD markers is consistent with previous study on cumin in which SCoT markers showed an efficient tool for analyzing genetic diversity (Vivodik *et al.*, 2017; Collard & Mackill, 2009).

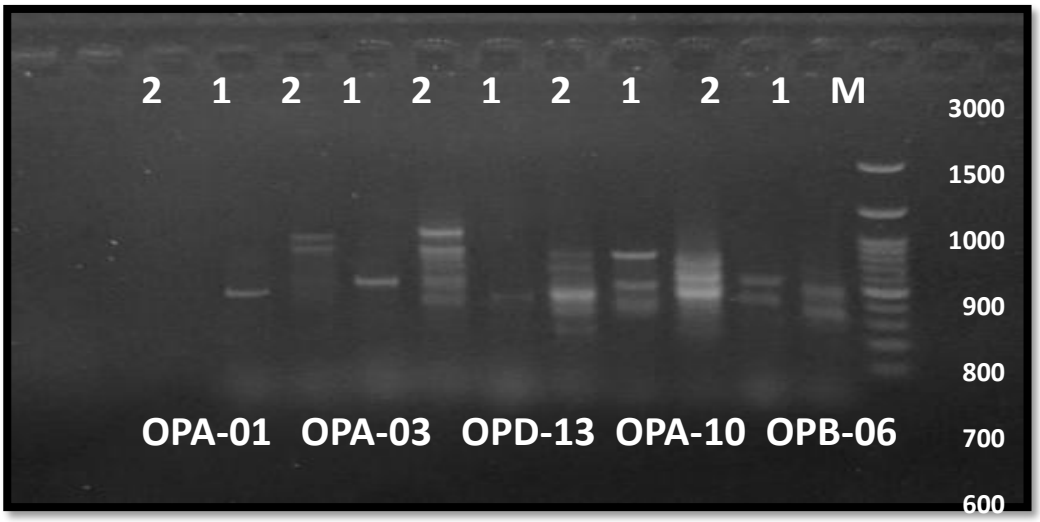


Figure (1): RAPD primer amplification products examples of sample 1 (local cumin) and sample 2 (black cumin). M: DNA ladder (100-1500 bp).

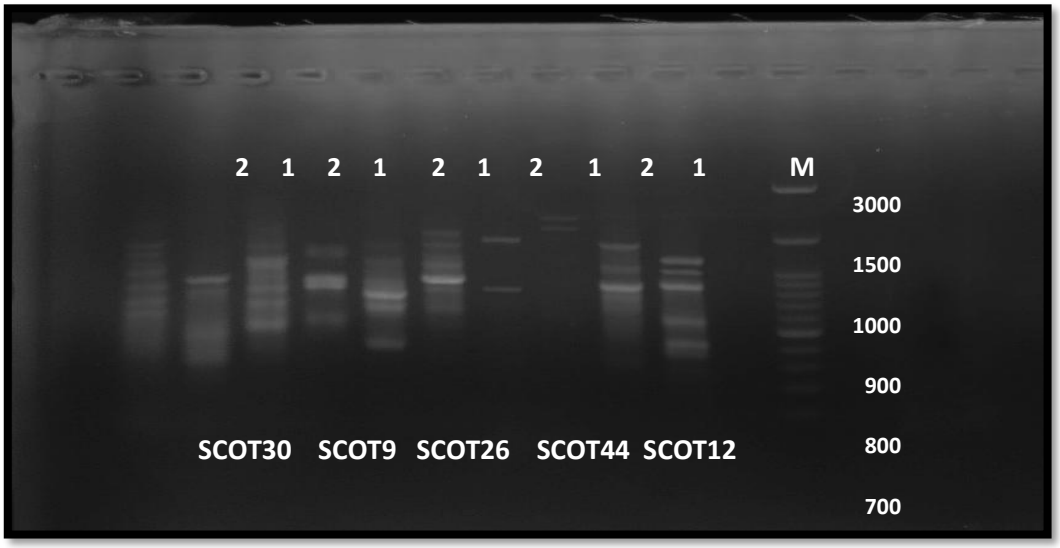


Figure (2): Amplification products of SCoT primers (SCoT12, SCoT44, SCoT26, SCoT9 and SCoT30) for samples 1 (local cumin) and 2 (black cumin). M: DNA ladder (100-3000 bp).

3.2. Essential Oil Analysis Using GC-MS

3.2.1. Essential Oil Constituents of *C. cyminum*

GC-MS analysis of the essential oil extracted from local cumin seeds revealed five major compounds (Table 3). The most common compound was cuminaldehyde at 42.43%, then γ-terpinene (25.12%) and β-pinene (12.23%), and finally p-cymene (10.97%) and limonene (9.93%). The grant concentration of these materials have recorded 864.7 mg/50 g of the sample, which is equivalent to 1.188 g/100 g (or 1.188%). These findings are in strong agreement with recent studies indicating that cuminaldehyde is the major constituent of cumin oil, followed by γ-terpinene and β-pinene (Acimovic *et al.*, 2024; Ouryemchi *et al.*, 2024).

Table 3: Essential oil constituents of *C. cyminum*

No.	Compound	Concentration (mg/50 g)	Percentage (%)
1	**Cuminaldehyde**	366.9	42.43%
2	**γ-Terpinene**	217.33	25.12%
3	**p-Cymene**	94.9	10.97%
4	**β-Pinene**	104.6	12.23%
5	**Limonene**	80.95	9.93%
	Total	**864.7 mg/50 g**	**≈100%**

3.2.2. Essential Oil Constituents of *B. bulbocastanum*

GC-MS analysis of the essential oil extracted from black cumin seeds revealed five major compounds (Table 4). The most common compound was thymol at 35.5%, then p-cymene (23.18%) and carvacrol (15.53%), and finally caryophyllene (14.75%) and β-myrcene (12.5%). The grant concentration of these materials recorded 1062.6 mg/50 g of the sample, which is equivalent to 2.125 g/100 g (or 2.125%). This large content of thymol and carvacrol distinguishes black cumin oil and responsible for its numerous biological properties (Al-Mansour *et al*., 2024; Dehghani *et al*., 2024).

Table 4: Essential oil constituents of *B. bulbocastanum*

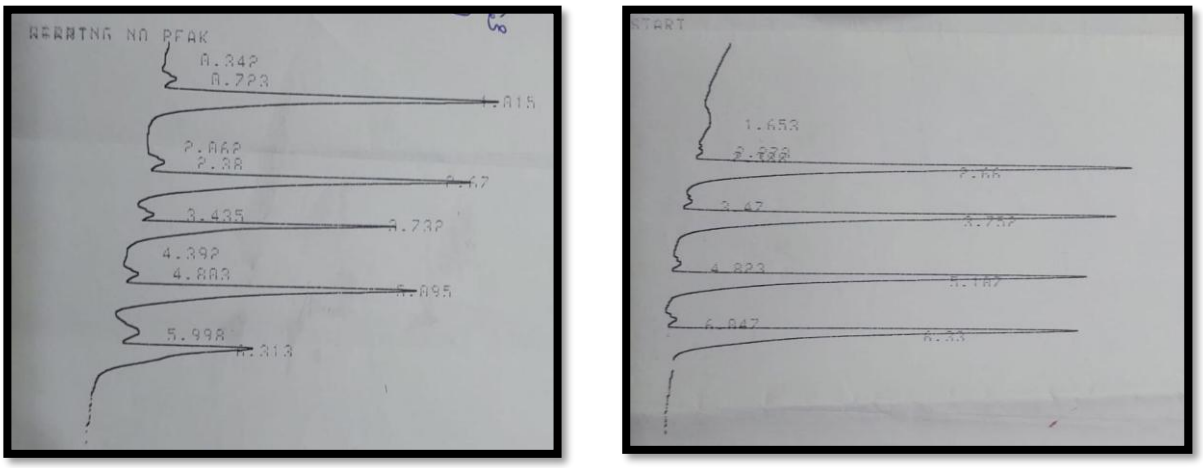
No.	Compound	Concentration (mg/50 g)	Percentage (%)
1	**Thymol**	359.6	35.5%
2	**p-Cymene**	254.7	23.18%
3	**β-Myrcene**	137.3	12.5%
4	**Caryophyllene**	140.2	14.75%
5	**Carvacrol**	170.82	15.53%
	Total	**1062.6 mg/50 g**	**≈100%**

3.3. Flavonoid Analysis Using HPLC

Identified and quantified by HPLC were four major flavonoid compounds (apigenin, kaempferol, rutin, and quercetin) in both species (Table 5, Figure 3). Concentrations of all studied compounds were generally similar between the two species. The local cumin had 607.44 µg/mL of total flavonoid and black cumin have 603.87 µg/mL total flavonoid. Apigenyn was the major compound in both species (187.49 and 188.0 µg/mL, respectively) followed by rutin, kaempferol and quercetin. The findings from these studies (see Table Table3) reveals that both species are rich in flavonoids with similar compositions, which may partly account for their strong antioxidant properties (Kashkolinia *et al*., 2024; Bendifallah *et al*., 2025).

Table 5: Flavonoid compound concentrations in *C. cyminum* and *B. bulbocastanum*

No.	Compound	Retention Time (min)	*C. cyminum* (µg/mL)	*B. bulbocastanum* (µg/mL)
1	**Apigenin**	2.66	187.49	188.0
2	**Kaempferol**	3.75	145.44	156.3
3	**Rutin**	5.01	162.7	159.3
4	**Quercetin**	6.33	111.59	100.27
	Total		**607.44 µg/mL**	**603.87 µg/mL**



A **B**

Figure (3): The HPLC chromatograms of flavonoid compounds in (A) local cumin *C. cyminum* and (B) black cumin *B. bulbocastanum*.

3.4. Physicochemical Properties of Essential Oils

Evaluation and presentation of essential oils basic physicochemical properties from both species (presented in Table 6). Characterized by cumin odor, both oils appeared as pale to golden yellow liquids. Values obtained for density and refractive indices were from 0.902 to 0.92 g/cm³ and 1.489 and 1.499, respectively which are comparable to those found for essential oils of the Apiaceae family (Shaaban Hamdy, 2023). Moisture content in both samples was low, at 0.7% in local cumin and 0.08% in black cumin, which exhibits good oxidative stability and oil quality. Black cumin produced 2.125% oil yield, where as local cumin produced the oil yield from 1.729%.

Table 6: Physicochemical properties of essential oils.

No.	Property	*C. cyminum*	*B. bulbocastanum*
1	Appearance	Pale to golden yellow liquid	Pale to golden yellow liquid
2	Odor	Characteristic cumin aroma	Characteristic cumin aroma
3	Density (at 20-25 °C)	0.902 g/cm³	0.92 g/cm³

No.	Property	*C. cyminum*	*B. bulbocastanum*
4	Refractive index (nD^{20})	1.489	1.499
5	Optical rotation	-5 to +5	-5 to +5
6	Moisture content	0.7%	0.08%
7	Yield (g/100 g)	1.729%	2.125%

DISCUSSION

Both RAPD and SCoT findings could unambiguously separate Cuminum cyminum from *B. bulbocastanum* in present research, confirming the robustness of molecular techniques e.g. RAPD and SCoT in determining genetic diversity among close relatives of Apiaceae (Collard & Mackill, 2009). Of the two systems for genetic diversity estimation, SCoT markers exhibited wider genetic diversity than RAPD markers in terms of the number of polymorphic bands (49 bands vs. 19 bands for RAPD). This result is expected in comparison with previous reports which have showed SCoT markers with ability of detecting genetic variation in the conserved start codon regions so these genetic markers are more reliable perform in the taxon and genetic studies than random RAPD markers (Vivodik et al, 2027). The genetic diversity documented here may offer a resource for hybridization and genetic improvement programs and emphasizes the need for conservation strategies for these species in Iraq and the neighboring region (Al-Mansour *et al*., 2024; Singh & Sengar, 2015).

Results of the GC-MS confirmed that cuminaldehyde was the predominent constituent of local cuminEO (42.43%) which was in agreement with those recent reports stating that cuminaldehyde contributes approximately between 25% to 45% of total cumin oil (Acimovic et al., 2024; Ouryemchi et al., 2024). Other major constituents included γ-terpinene (25.12%) and β-pinene (12.23%) which are both essential for contributing to the signature scent of the oil and its antimicrobial properties; this synergy between cuminaldehyde and γ-terpinene was found to have highlighted effects on antifungal activity (Msegued Ayam et al., 2025). Black cumin essential oil was characterized with high thymol content being 35.5% in greater amounts than reported earlier for *B. persicum* (Dehghani et al., 2024), and with p-cymene (23.18%) and carvacrol (15.53%) as other major components. Carvacrol and thymol have been reported to be associated with strong antimicrobial, anti-inflammatory, and antioxidant activities when co-occurring (Yilmaz & Turan, 2025; Ari et al., 2024). Based on species comparison, it is possible to expect fundamental differences: for example, because cuminaldehyde predominates local cumin oil, on the contrary, in black cumin oil, both thymol and carvacrol predominate, which explains the variability in their therapeutic properties, because higher phenol content in oils from *C. maritimum* and *C. okamurae* has been connected with stronger antioxidant activity (Kashkolinia *et al*., 2024; Dehghani *et al*., 2024).

Samples from both species demonstrated remarkable similarity in their concentrations of flavonoids (607.44 µg/mL in local cumin versus 603.87 µg/mL in black cumin), which supports the premise of analogous secondary metabolic pathways as local cumin and black cumin belong to the same family (Bendifallah et al., 2025) [3]. Both species were dominated by apigenin, which demonstrates strong antioxidant and anti-inflammatory capabilities

(Kashkolinia et al., 2024). Our results shows a multilayered relationship between genetic diversity and chemical composition: genetic analyses detected substantial variation, whereas flavonoid content variation was less pronounced, supporting the hypothesis that flavonoid biosynthesis genes are highly conserved. Essential oil compositions, however, exhibited more extreme differences, correlating with more diverse distribution of genes associated with terpene and phenol biosynthesis (Bendifallah *et al*., 2025).

Fifty-three percent of the variance in sensory characteristics were due to physicochemical properties, where high oil quality were explained by very low moisture content (0.7% & 0.08%) further confirming high quality of oil from effective extraction and storage (Acimovic et al., 2024). The density and refractive index values were in the normal range for Apiaceae oils, which confirmed purity (Shaaban Hamdy, 2023). The yield of oil from black cumin (2.125%) was almost double that of local cumin (1.729%), which is an important economic advantage. Results form good scientific evidence for traditional uses of both species in Iraqi folk medicine (Hama & Omer, 2024), and could be useful for manufacturing pharmaceutical, food and cosmetic products. The genetic variation includes potential for improved genotypes is apparent, which can be exploited through breeding programs (Govindaraj *et al*., 2015).

CONCLUSION

The study could demonstrate that *Cuminum cyminum* and *Bunium bulbocastanum* are widely divergent genetically and chemically, identifying SCoT as a more effective genetic marker than RAPD for genetic diversity characterization. Essential oil extracts from aboveground parts of *B. bulbocastanum* (CV: 49.9-62.7%) and *C. cyminum* (CV: 77.8-85.1%) indicated the existence of chemotypes, with cuminaldehyde being the primary oil component of the former species (42.43%), and thymol (35.5%) and carvacrol (15.53%) were exhibited the characteristics of the latter. Both species showed a high and similar flavonoid content (607.44 and 603.87 µg/mL, respectively), with apigenin being the major compound, confirming the use of these plants in folk medicine for their antioxidant properties. The results offer a basis for the conservation, cultivation, and commercial use of these bioactive medicinal plants in various industries such as pharmaceuticals, nutrition, and cosmetics.

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