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Optimization of oleoresin extraction from coriander seeds and isolating fatty acids by column chromatography

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ABSTRACT

Coriander (*Coriandrum sativum* L.) is widely used as a flavor enhancer in food and medicinal products due to its rich content of bioactive compounds, including fatty acids, polyphenols, and essential oils. Among these, linoleic acid a predominant fatty acid in coriander oleoresin imparts significant medicinal properties, such as antioxidant, antimicrobial, anti-inflammatory, and hypolipidemic activities. In this study, we optimized oleoresin extraction from coriander seeds at room temperature using maceration, sonication, and a combined ultrasound-maceration method. The hyphenated ultrasound-maceration method (15 min sonication at 50% power followed by 24 h maceration) yielded the highest extraction efficiency (4.67%), while 24 h maceration alone yielded 3.92%. Given the marginal yield difference, maceration was deemed more cost-effective for large-scale applications. The overall extraction curve (OEC) for maceration revealed that 12 h extraction provided an optimal balance between yield (3.78%) and total phenolic content (27.88 mg GAE/g extract), making it the preferred duration. For fatty acid isolation, silica gel column chromatography with gradient elution (96% hexane/4% ethyl acetate) successfully separated a fatty acid-rich fraction (0.272 g). Structural characterization via ^{1}H NMR and GC-MS confirmed the presence of linoleic acid, one of the most abundant fatty acids in coriander oleoresin.

Keywords: *Coriander, Fatty acid, Maceration, Extraction, Linoleic acid*

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Introduction

Oleoresin, commonly marketed as spice drops, are concentrated extracts obtained from plant, spices and herbs (1). They are comprised of volatile and non-volatile compounds that making them more complicated extracts (2). Oleoresins are generated by the epithelial cells that form the lining of the resin ducts (3). They are abundant in active compounds and can provide antimicrobial, antioxidant, and anti-inflammatory activities. Due to rising health concerns, consumers are gradually looking for natural products with nutritional benefits so Oleoresins use as substitutes for synthetic chemical additives (4). Paprika and turmeric oleoresins are examples for coloring applications, while oleoresin from coriander and cinnamon is typically used due to aroma and taste (4).

Coriander (*Coriandrum sativum* L.) is an aromatic Mediterranean plant mainly cultivated for its seeds. It belongs to family Umbelliferae with height of 20 to 70 cm, and a narrow and spindle-shaped root. the leaves are oval light green and branched at the bottom of the plant which have long been used as a flavor enhancer in food and various medicinal products (5, 6). The plant typically matures within 90–120days. For oil extraction harvesting should occur when approximately 60% of the fruits have turned brown or central umbels attain a yellow color, because at this stage the contents of the volatile oil are at their maximum level (7). The use of coriander seeds can be traced back to 1000 BC, but it has been supposed that they were cultivated for the first time over 2000BC in the Hanging Gardens of Babylon (8). A literature survey demonstrates that The coriander offers considerable antioxidative, antimicrobial, and anti-inflammatory properties (9) and has been known due to its exceptional phytochemicals that are not common in other plant (10). Essential oils and fatty acids extracted from its dried fruit can be used commercially in pharmaceutical, food and beverages, plastics, cosmetics, fragrance and detergent industries(10). Coriander seed oil is particularly rich in the less-common monounsaturated isomer of oleic acid, *i.e.*, petroselinic acid (C_{18}:1n12) (11). Coriander seeds are rich in fatty acids, The main fatty acids identified in coriander seeds are petroselinic, linoleic, oleic, and palmitic acids (10). And hence, the fatty acids in coriander are of significant interest for industrial applications. It is accepted that column chromatography offers possibility for fractionation of the extracts, therefore, we decided to use column chromatography for separation of oleoresin fatty acids of coriander seeds.

Extraction is a crucial step in natural compounds processing that could be optimize to being more efficient to achieve better yield and targeted compounds in extracts (12). Hence it is important to choose the best extraction method. Coriander seed oleoresin can be prepared by numerous techniques such as maceration, sonication, using hydrothermal autoclave and high shear mixer or combination of several methods. The solvent extraction is more cost effective and efficient technique, consequently this study seeks to optimize coriander oleoresin extraction and isolate its fatty acids by column chromatography. in this research phytochemical analysis of coriander oleoresin was performed using ^{1}H NMR and GC-MS.

Material and methods

Reagents and materials

Sodium carbonate ($Na2CO_3$), Folin-Ciocalteu, potassium hydroxide (KOH), Boron trifluride (BF3) was purchased from Sigma-Aldrich (Germany). Gallic acid (GA) was obtained from Phytolab (Germany) and Phytopurify (Chengdu, Sichuan, China). Also, organic solvents were supplied by Samsung (South Korea). Solvents for nuclear magnetic resonance (NMR) spectroscopy were obtained from Armar Chemicals (Döttingen, Switzerland).

Extraction process

Coriander seeds were collected from Sahneh (Kermanshah Province, Iran) and transported to the Medicinal Plants and Drug Research Institute at Shahid Beheshti University, Tehran. The seeds were ground and sieved to obtain particle sizes ranging from 2 mm (sieve No. 10) to 106 µm (sieve No. 140) to optimize extraction efficiency. For maceration experiments, four identical samples were prepared, each containing 10 g of seed powder and 50 mL of 96% ethanol (solvent-to-feed ratio 5:1) in 100 mL Erlenmeyer flasks. Each flask was equipped with a 2 cm magnetic stir bar, sealed with aluminum foil to prevent solvent evaporation, and agitated at 250 rpm on a magnetic stirrer. Extraction was conducted at 25°C for four-time intervals (2, 8, 12, and 24 h) to establish the extraction kinetic profile. Post-extraction, the solutions were filtered and concentrated using rotary evaporation. Extraction yields were calculated gravimetrically. The crude extracts were then centrifuged (1,000 rpm, 1 h), yielding two distinct layers: an upper oleoresin fraction and a lower viscous layer, which were separated and stored at 4°C for subsequent analysis. The optimal extraction time was determined by monitoring the extraction yield kinetics (OEC). Figure 1 shows the extraction process schematically.

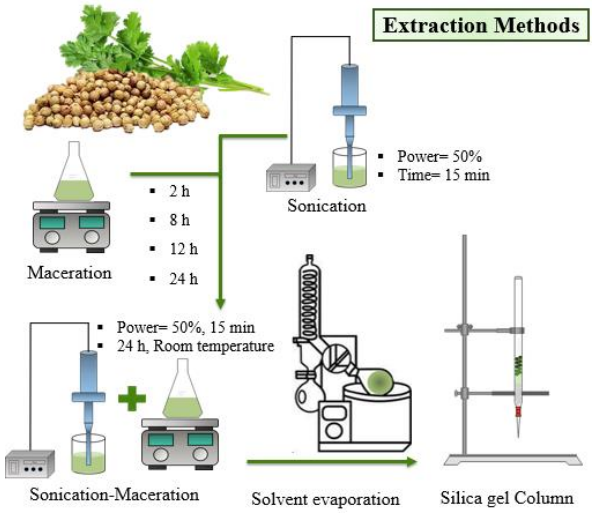

Fig. 1. Diagram showing extraction procedure.

Extraction yield (%) = $\frac{Dried\ extract\ weight}{coriander\ seed\ powder(10g)}$×100

Overall Extraction Curve (OEC) diagrams are used to examine how factors such as time, solvent amount, or other parameters affect extraction. They also provide information about the extraction kinetics. These diagrams consist of three regions: FER, CER, and DC.

1-CER (Constant Extraction Rate): The region represents where the extraction of active compounds occurs more easily and at a constant rate.

2-FER (Falling Extraction Rate): The transition phase where extraction becomes somewhat more difficult and extraction rate begin to decline.

3-DC (Diffusion Controlled): The region where changes in extraction rate are minimal, and extraction occurs mainly through diffusion(13).

Ultrasound Assisted Extraction

UAE was performed 15 minutes at 50% power as previously (Tacchini et al., 2019) was used(14). the dried powdered of coriander seeds (10g) were extracted with 50 mL of 96% ethanol with power 50% for 15 minutes. subsequently the obtained extract was

filtered and dried with rotary evaporator and then extraction yield was calculated.

Total phenolic content determination

The total phenolic content of the oleoresin was determined using the Folin-Ciocalteu (F-C) method (15). Exactly 125µL of diluted F-C (1:9 in water) and 100µL of sodium carbonate (7.5% w/w) solution were added to 25µL of diluted sample (1000ppm). then, the absorption of the sample was read at 760 nm after two hours by a multifunctional microplate reader. The results were announced in mg gallic acid (GA)equivalent per gram of sample dry weight (15).

Column chromatography methodology

Column chromatography was employed for the separation and purification of compounds from coriander oleoresin. The glass chromatography column (2.42 cm outer diameter) was packed with silica gel (70-230 mesh, 63-200 µm) to a height of 17.5 cm, providing a 100 mL bed volume. The sample-to-stationary phase ratio was maintained at 1:30 (1 g oleoresin:30 g silica). Prior to column separation, preliminary thin-layer chromatography (TLC) screening was conducted to optimize the solvent system, which led to the selection of hexane, ethyl acetate, and methanol as mobile phases. Gradient elution was initiated with 100% hexane at a flow rate of 3 mL/min. The polarity was systematically increased by introducing ethyl acetate (4-96% gradient), followed by methanol. Notably, fatty acids were effectively isolated using 96% hexane/4% ethyl acetate (Table 1). The target fraction was collected and subjected to structural characterization using ^{1}H NMR spectroscopy and gas chromatography-mass spectrometry (GC-MS) for comprehensive analysis.

Table 1. Gradient elution of chromatography column

Solvent composition	Solvent ratio	Fraction amount(g)
Hexane	100	0.0039
Hexane-ethyl acetate	98-2	0.0022
Hexane-ethyl acetate	96-4	0.272
Hexane-ethyl acetate	94-6	0.0939
Hexane-ethyl acetate	92-8	0.0642
Hexane-ethyl acetate	90-10	0.0822
Hexane-ethyl acetate	88-12	0.0542
Hexane-ethyl acetate	86-14	0.0173
Hexane-ethyl acetate	84-16	0.0152
Hexane-ethyl acetate	82-18	0.0061
Hexane-ethyl acetate	80-20	0.0049
Hexane-ethyl acetate	70-30	0.0054
Hexane-ethyl acetate	60-40	0.1241
Hexane-ethyl acetate	50-50	0.0075
Hexane-ethyl acetate	40-60	0.0051
Ethyl acetate	100	0.0055
Ethyl acetate-methanol	50-50	0.1111
Methanol	100	0.0536

GC-MS analysis

For identification of fatty acids, gas chromatograph-mass spectrometer (GC-MS), equipped with a DB-5 column (30m × 0.25 mm; 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 22 mL/min. The injector and detector temperatures were set at 250°C, and the injector was operated in split mode with a split ratio of 20:1. The temperature program for the column was as follows: the oven temperature started at 100°C and held for 5 minutes, then increased at a rate of 4°C/min until reaching 238°C, where it was maintained for 20 minutes. The mass spectrometer was equipped with an

electron ionization (EI) source and a quadrupole mass analyzer. The ion source temperature was set at 200°C. After the instrument was prepared, 0.2 µL of the prepared fatty acid methyl ester sample was injected for analysis.

Derivatization process

A sensitive gas chromatography-mass spectroscopy (GC–MS) approach was employed to evaluate the fraction containing fatty acids obtained from column chromatography. For this purpose, the fatty acids must first undergo a derivatization process to convert them into their methyl ester forms, thereby increasing their volatility for GC-MS analysis.

For derivatization of fatty acids, after weighing the sample:

- Add 100 mg of potassium hydroxide (KOH) for every 100 mg of fatty acid.
- Then, add 8 mL of methanol per 100 mg of fatty acid.
- Next, add 2 mL of boron trifluoride (BF3), which acts as a catalyst, for every 100 mg of fatty acid.

The resulting solution is placed in a water bath at 60°C for one hour and stirred every 10 minutes. During this process, the hydroxyl groups (OH) in the fatty acids are converted to methyl esters (OMe). After the sample reaches room temperature, 10 mL of distilled water is added per 100 mg of fatty acid, followed by the addition of 1 mL of hexane per 100 mg of fatty acid. The soluble compounds dissolve in the hexane, forming the upper organic phase. The samples are then centrifuged at 4000 rpm for 10 minutes. After centrifugation, the upper layer containing hexane and the fatty acid methyl esters is separated and injected into the instrument for further analysis(16).

Results and Discussion

Extraction yield calculation

The maceration extraction yields are presented in Table 2. The yield increases rapidly in the first 2 hours, reaching 1.42%, indicating efficient initial solubilization of readily available compounds. From 2 to 8 hours, the yield rises more significantly to 3.15%, suggesting progressive extraction of less accessible components. Beyond 8 hours, the rate of extraction slows, with the yield reaching 3.8% at 12 hours before stabilizing, likely due to solvent saturation or depletion of extractable compounds. This plateau suggests that extending maceration beyond 12 hours offers negligible additional yield.

Table 2. The comparison of extraction yield in different maceration samples

Sample name	Extraction time(h)	Yield%
Mac1	2	1.42
Mac2	8	3.15
Mac3	12	3.80
Mac4	24	3.92

The extraction yield, as shown in Fig. 2, exhibits a rapid increase during the first 8 hours, rising from 1.42% to 3.15%. Beyond 8 hours, the rate of extraction slows, with only a slight increase observed between 8 and 12 hours. From 12 to 24 hours, the yield stabilizes at an average of 3.80%, indicating that equilibrium has been reached and further extraction time does not significantly enhance yield.

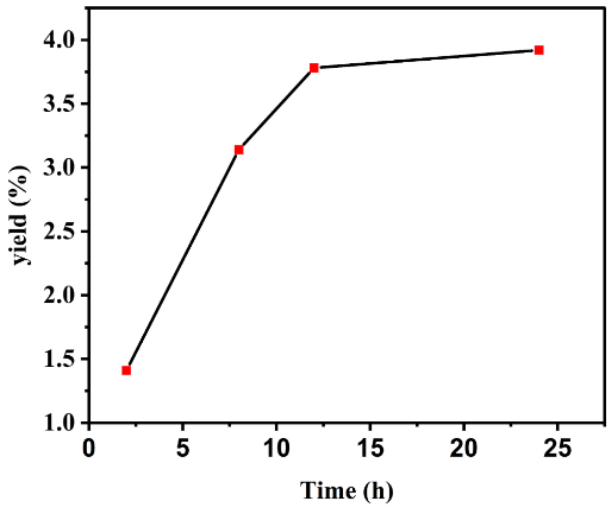

Fig. 2. OEC diagram of coriander oleoresin

Furthermore, the total phenolic content analysis (Tables 3 & 4) corroborates these findings. Based on the results, 12-hour maceration was selected as the optimal extraction time, yielding 3.80% extract with a total phenolic content of 27.88 mg GAE/g. For comparison, alternative methods ultrasonic probe extraction alone and sonication pretreatment followed by maceration achieved yields of 2.51% and 4.67%, respectively. The higher extraction yield observed in sonic-assisted maceration (4.67%) compared to conventional maceration (3.92%) may result from ultrasonic pretreatment, which enhances mass transfer and cell wall disruption. additionally a sonicator with 50 % power was applied to the solid-liquid mixture for 15 min to extract oleoresin as mentioned in Table 4. However, maceration remains the more cost-effective method, as the marginal yield improvement from sonication does not justify the additional energy and equipment costs in large-scale applications.

Table 3. Total phenolic content (TPC) comparison across maceration samples (mg GAE/g extract)

Sample name	Extraction time(h)	TPC (mg GA.g^{-1})
Mac1	2	16.21
Mac2	8	26.78
Mac3	12	27.88
Mac4	24	28.96

Table 4. Total phenolic content (TPC) comparison across sonication samples (mg GAE/g extract)

Sample name	Extraction technique	TPC (mg GA.g^{-1})
Sonic	t:15min, p=50%	11.99
sonic-maceration	t:15min, p=50%+mac(24h)	24.28

Column-chromatography

After loading the column with maceration-derived oleoresin and performing gradient elution (Table 1), 18 fractions were collected. Fractions exhibiting identical TLC profiles were pooled for further analysis. Fraction 3 (0.272 g), selected based on its distinct TLC banding pattern, was hypothesized to contain the fatty acid constituents of the oleoresin. This fraction was subsequently analyzed by GC-MS and ^{1}H-NMR spectroscopy (Fig. 3) for structural confirmation. The

gradient elution 96% hexane 4% ethyl acetate isolates fatty acids proved based on the ^{1}H NMR data.

Fig. 3. Fractionation of coriander oleoresin by silica gel column chromatography and eluted fractions.

The ^{1}H-NMR spectrum (Fig. 4) showed no acidic proton signal (~12 ppm), confirming the absence of free fatty acids, with only a residual solvent peak at 7.2 ppm ($CDCl_3$). Six distinct signal groups were identified: 0.89 ppm (a, terminal CH_3), 1.67 ppm (b, β-CH_2 to carbonyl), 2.06 ppm (c, allylic CH_2), 2.34 ppm (d, α-CH_2 to carbonyl), 2.79 ppm (e, bis-allylic CH), 4.14–4.32 ppm (f/g, glycerol sn-1/sn-2 protons), 5.29 ppm (h, glycerol sn-3 proton), and 5.35 ppm (i, olefinic CH=CH). These patterns, combined with GC-MS data, conclusively identified linoleic acid in triglyceride form as the dominant fatty acid in the coriander oleoresin.

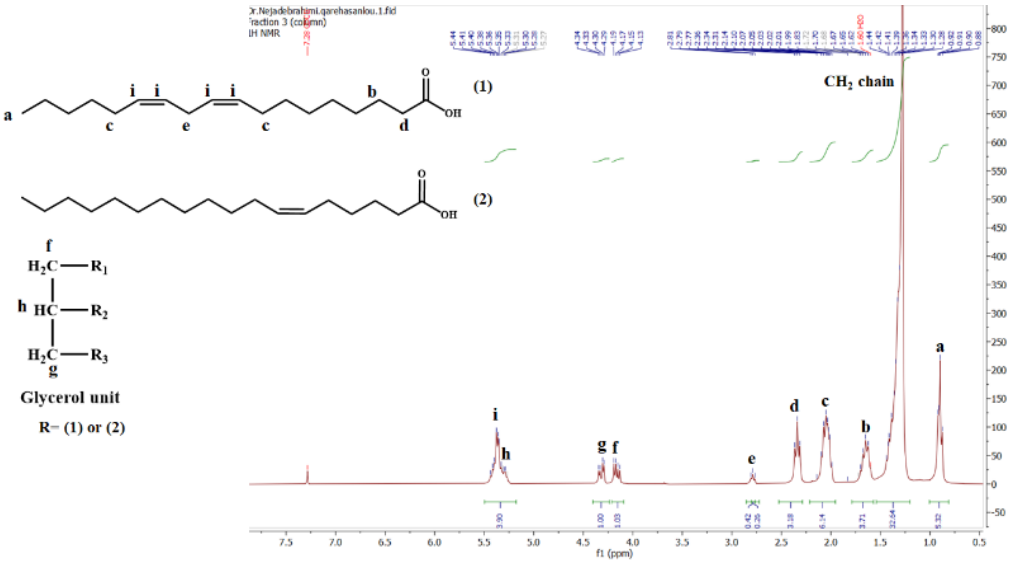

Fig 4. NMR spectra of coriander oleoresin.

GC-MS analysis

A very sensitive and selective gas chromatography-mass spectroscopy approach (GC–MS) was employed to evaluate the fatty acid fraction of coriander oleoresin compositions (Fig. 5). Linoleic acid was identified with a peak area percentage of 28.82%, while oleic acid was detected with a peak area percentage of 56.82.%

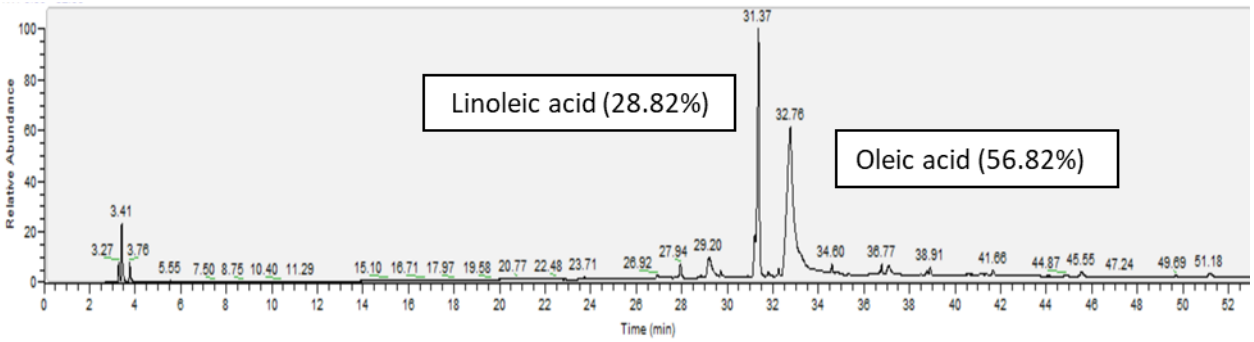

Fig 5. GC-MS chromatogram of the fatty acids fraction of coriander oil.

Conclusion

Coriander seeds are rich in fatty acids which are of significant interest for industrial applications.in this study OEC curve was designed and provided an optimal balance between yield **(3.78%)** and total phenolic content **(27.88 mg GAE/g extract),** and making 12 h maceration the preferred extraction technique and time. Moreover, the obtained oleoresin was used to isolates its fatty acids with column chromatography. for this purpose, the gradient elution 96% hexane 4% ethyl acetate used to isolate fatty acids fraction (0.272g) proved based on the ^{1}H NMR data and linoleic acid, which is the one of abundant fatty acid in coriander oleoresin, was identified in fatty acid fraction.

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Authors' Contributions

Nessa Gharehassanloo: Conceptualization, Investigation, Formal analysis, Writing-original draft. **Atefeh Safari:** Methodology, Formal analysis, Visualization. **Mahshad Shahriari:** Formal analysis, Data Curation, Visualization, Writing-review and editing. **Samad Nejad Ebrahimi:** Supervision, Methodology, Project administration, Visualization, Writing-review and editing. **Ali Sonboli:** Conceptualization, Supervision, Formal analysis, Writing-review and editing.

Declaration of Interest

The authors of this article declared no conflict of interest.

Ethical Considerations

All ethical principles were adheried in conducting and writing this article.

Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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