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Chemical Composition and Antioxidant Activity of Essential Oil of *Coriandrum sativum* **L. Seeds Cultivated in Afghanistan**

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Authors' contributions

This work was carried out in collaboration between both authors. Author SJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors YM and SJ managed the analyses of the study. Authors SJ and YM managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

Coriandrum sativum L. is an aromatic plant belonging to the Apiaceae (Umbelliferae) family, originating in the Mediterranean region. *C. sativum* is widely cultivated worldwide and has nutritional and medicinal values. The *C. sativum* seeds contain an essential oil that is used in different industries like pharmaceuticals, cosmetics, and food. This study aims to extract, analyze, and evaluate the antioxidant activity of *C. sativum* seed essential oil cultivated in Afghanistan. The essential oil was extracted by hydro-distillation (HD) extraction and analyzed by High-performance thin-layer chromatography (HPTLC). The HD extraction provided an essential oil yield of 0.16 % (v/w). Also, HPTLC analysis of the essential oil determined two components of the oil, linalool and myrcene. The quantification of linalool content and HPTLC method validation were determined using densitometric analysis. As a result, the linalool content was identified 60.06 %, and the HPTLC method proved as a valid method for analysis of the essential oil. Furthermore, the antioxidant activity of the essential oil was determined using DPPH radical scavenging assay and reported as IC₅₀. The ascorbic acid utilized as the positive control, and the antioxidant activity of the

essential oil evaluated in comparison with ascorbic acid. The IC_{50} of ascorbic acid and the essential oil were determined 0.02 ± 0.0004 (mg/ml) and 21.05 ± 0.284 (mg/ml), respectively. Thus, the essential oil showed lower antioxidant activity than ascorbic acid. This study is the first report on the extraction, chemical composition, and antioxidant activity of *C. sativum* seed essential oil cultivated in Afghanistan.

Keywords: Coriandrum sativum L.; essential oil; hydrodistillation; high-performance thin-layer chromatography; DPPH free radical; antioxidant activity; Afghanistan.

1. INTRODUCTION

Coriandrum sativum L. is a well-known aromatic and medicinal plant, which belongs to the Apiaceae (Umbelliferae) family and popularly known as coriander [1,2]. *C. sativum* is an annual herb originating in the Mediterranean region, cultivated since ancient times. This plant is widely grown in Russia, Central Europe, North Africa, and Asia [3,4]. *C. sativum* possesses both nutritional and medicinal properties among the most widely used medicinal plants [5]. The dried seeds are employed to flavor pickle spices, seasonings, curry powders, sausages, and pastries, biscuits, buns [6]. Additionally, as a medicinal plant, *C. sativum* has been reputed to have many medical uses. Several therapeutic properties of *C. sativum* reported, such as hypoglycaemic, anti-inflammatory, hypolipidaemic, analgesic, sedative, anxiolytic, fttantimutagenic, antihypertensive, diuretic, antioxidant, antimicrobial, carminative, antispasmodic, relaxant, anthelmintic, antiprotozoal, anticancer, and anti-fertility [4,7,8,9].

In Afghanistan, *C. sativum* is used as a contraceptive agent and memory strengthener in traditional medicine. The seeds are used as a spice and condiment for cooking and flavoring beverages [10,11]. The local name of *C. sativum* is Gashneez and is cultivated in 65 regions in different provinces of Afghanistan. *C. sativum* seeds account for 0.5% of exports of medicinal and aromatic plants of Afghanistan. For example, 223.8 tons of seeds worth \$ 458.3 thousand had exported in 2016. In addition, *C. sativum* seeds are ranked 15th among the 25 most important Afghan medicinal and aromatic plants in terms of socio-economic importance and exports [12].

Different parts of the *C. sativum* contain essential oil such as leaves, flowers, stems, seeds, roots, and bark; but, the essential oils of different parts vary in compositions. For example, the main compositions of fresh herb essential oil are aliphatic aldehydes (mainly C10-C16 aldehydes) with a fetid-like aroma. However, linalool, oxygenated monoterpenes and monoterpene hydrocarbons are the main components of seeds' essential oil [3,13]. Based on reports, the seeds contain an essential oil that varies from very low to a maximum amount (0.03%- 2.7%). The *C. sativum* seed essential oil has a place among the 20 significant essential oil in the world market [6,14]. The main component of the *C. sativum* seed essential oil is linalool that makes up 50-70% of the essential oil. Other significant components of the essential oil include geranyl acetate, limonene, camphor, geraniol, borneol, citronellol, pinenes, γ-terpinene, myrcene, camphene, phellandrenes, α-terpinene, limonene, cymene [1,3,15]. Furthermore, *C. sativum* seed essential oil and extract have antibacterial, antioxidant, anticancer, and antimutagenic activities [9].

There is a lack of equipment such as HPLC in resources limited countries. Moreover, in developing countries, the high cost of HPLC grade solvents and columns and consumption of solvents can significantly influence on timely release of laboratory results for operation. Therefore, there is a need for methods to ease and increase the speed of analysis with eligible costs [16]. High-performance thin-layer chromatography (HPTLC) has become a routine analytical technique due to its simplicity and widely used to evaluate phytochemicals of botanicals [17]. HPTLC is a simple, rapid, and cost-effective technique for analyzing several samples at a time [18]. The advantages of HPTLC involve the usage of a minimum sample, no prior treatment for solvents like filtration and degassing, and low analysis time. Moreover, this method allows parallel treatment of multiple samples during chromatography, and there is no interference from the previous analysis (fresh stationary phase and mobile phase for each analysis) [16,17,19]. In addition, the HPTLC technique is used for quality control of raw materials and standardization of polyherbal formulations [17].

C. sativum essential oil has shown more significant activity against 1,1-diphenyl-2 picrylhydrazyl (DPPH[®]) free radicals among several essential oils, indicating that the essential oil is a great source of bioactive compounds and has a considerable antioxidant activity as a part of dietry regime [20]. The DPPH assay is a commonly practiced method to evaluate an antioxidant molecule's free radical scavenging potential, considered a valid and convenient colorimetric method [21,22]. DPPH[•] is a stable radical having deep purple color and strong absorption at 517 nm. This assay depends on donating an electron or hydrogen atom by an antioxidant compound and converting the DPPH[®] radical to a more stable molecule. As a result, the purple color changes and yellow-colored diphenylpicrylhydrazine is formed which color change is observed by a spectrophotometer [21] [23,24]. The DPPH assay results show as SC_{50} or IC_{50} (the concentration of antioxidant causing inhibition of 50% DPPH● radicals) or as %scavenging of DPPH● at a fixed antioxidant concentration for all the samples [24].

The present study aimed to evaluate *C. sativum seed* essential oil antioxidant activity and develop an HPTLC method with the support of an image processing software to determine phytochemicals and quantify linalool as the main phytochemical of the essential oil. Moreover, the HPTLC method is validated based on the International Conference on Harmonisation (ICH) guidelines.

2. MATERIAL AND METHODS

2.1 Plant Material and Chemicals

The dried Seeds of *C. sativum* L. were obtained from a local market in Kabul. Fig. 1 shows *C. sativum* seeds.

Fig. 1. *C. sativum* **L. seeds purchased from a local market in Kabul, Afghanistan**

The HPTLC Silica gel 60 F254 (20*20) from Merck was used for chromatographic analysis of *C. sativum* essential oil. Moreover, linalool standard was purchased from TCI (Japan), while myrcene was provided from ACROS (Japan). *p*anisaldehyde used for derivatization in HPTLC analysis was procured from Kanto (Japan). Also, ascorbic acid and Tris base were obtained from Wako (Japan), but DPPH radical was purchased from TCI (Japan) for antioxidant measurement.

Solvents including ethanol (99.5%), hydrochloric acid, toluene, ethyl acetate, and sulfuric acid were provided from Wako (Japan), acetic acid was purchased from Kanto (Japan). All reagents were analytical grade.

2.2 Essential Oil Extraction

The extraction was performed according the method described by Faqeryar et al. with slight changes [25]. Essential oil extraction was performed by hydro-distillation. A 120 g of *C. sativum* dried seeds were weighed and subjected to hydrodistillation for 4h using a Clevenger type apparatus. The extracted essential oil was kept in an amber-colored glass vial in the refrigerator for further analysis.

2.3 HPTLC Analysis

HPTLC analysis was carried out based on methods described by Faqeryar et al. [25] and Alqarni et al. [26] with small modifications.

2.3.1 Preparation of sample and standard solutions

The sample solution was prepared by diluting 20 µL of *C. sativum* essential oil in 1 mL of toluene. Linalool and myrcene standard solutions were made by dissolving 5 μ L and 100 μ L of each in 1 mL toluene, respectively.

2.3.2 Chromatographic condition

Chromatographic analysis of *C. sativum* essential oil was performed utilizing a 10*10 cm HPTLC Silica gel 60 F254, aluminum plate (Merck, Darmstadt, Germany). The essential oil of *C. sativum* and standards were loaded in the HPTLC sheet using 2 µL capillary tubes. Spotting was performed at a 1 cm distance from the plate bottom and 1 cm distance from the plate side edges, then the plate was developed in the mobile phase up to 8.5 cm in a twin trough glass chamber (CAMAG). A mixture of toluene- ethyl

acetate (95:5) was used for development. The chamber was saturated by the mobile phase vapor for 20 min before the development. Moreover, a redeveloping method was used for better separation of *C. sativum* essential oil compositions.

2.3.3 Derivatization

The spots on HPTLC plates were detected using the *p*-anisaldehyde derivatizing reagent. The *p*anisaldehyde reagent was prepared by adding 2 mL glacial acetic acid, 185 mL of ethanol (95%), and 10 mL of concentrated sulfuric acid to 5 mL p-anisaldehyde in chronological order. The plates were immersed in the derivatization solution. Then, the HPTLC plates were air-dried and heated for 5 min at 100ºC on a hotplate. Finally, the visualized plates were scanned (Canon, LiDE220), and the scanned images of HPTLC plates were saved in JPEG format for further analysis.

2.3.4 Identification and quantification

The scanned images of HPTLC plates were used for qualitative and quantitative analysis. An image processing software (ImageJ software) was used for image processing and densitometric analysis to determine and quantify compositions of *C. sativum* essential oil. ImageJ can be freely downloaded and is available for both PC and MAC from the U.S. National Institute of Health (NIH) [27]. The identification of *C. sativum* essential oil was performed by comparing colors and retention factor (Rf) values of sample and standards spots. Also, quantitative analysis was carried out to quantify linalool as the main component of the essential oil. The linalool content was determined by using the standard calibration curve established with a concentration range of 2.15-12.9 µg/spot.

2.3.5 Validation of HPTLC method

The International Conference on Harmonisation guidelines was followed to validate the proposed HPTLC method regarding linearity, selectivity, sensitivity, precision, accuracy, robustness, and ruggedness.

2.3.5.1 Calibration curve and linearity

As mentioned previously, the linalool content was assessed by using the standard calibration curve established with a concentration range from 2.15 to 12.9 µg/spot. The standard solutions were loaded using capillary tubes on the HPTLC plate with a 12 mm distance between spots. The calibration curve was obtained by plotting each concentration peak area against the concentration of spotted linalool.

2.3.5.2 Selectivity and sensitivity

The selectivity of the HPTLC proposed method was assessed by analysis of standards and components of the essential oil. Accordingly, the retention factors (Rf) of standards were compared with the components containing in the essential oil.

The method's sensitivity was ascertained using the limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were determined using these formulae (3.3 ∂/s) and (10 ∂/s), respectively, where ∂ is the standard deviation (SD) of response at low concentrations and s is the slope of the calibration curve.

2.3.5.3 Precision

The precision of the method was evaluated by intra-day and inter-day variation studies. The intra-day and inter-day variation were determined by analyzing the standard solution of linalool (2,4,6 µg/spot) on the same day and different days. The results of the evaluation were expressed as relative standard deviation (RSD%).

2.3.5.4 Accuracy

Accuracy was examined by a recovery (%) study. The recovery study was performed by spiking the pre-analyzed sample with standard linalool at four different concentrations (0%, 50%, 100%, and 150%) of the analyte. The results of triplicate tests were reported as % recovery and RSD% for each concentration.

2.3.5.5 Robustness

The robustness was performed to evaluate the effect of small changes in the chromatographic method parameters on the results. The method's robustness was carried out by changing the mobile phase polarity at a linalool standard concentration level of 2 µg/spot. The result was expressed as RSD% of peak area.

2.3.5.6 Ruggedness

The ruggedness of the proposed method was defined by spotting the linalool standard (2 µg/spot) using the same equipment and conditions with different analysts.

2.4 DPPH Radical Scavenging Assay

The DPPH radical scavenging assay was carried out using the method described by Shimamura et al. with slight modifications [28]. Concisely, 2 mL of 0.2 mM DPPH[•] ethanolic solution was added to 400 µL various concentration of ethanolic solution of *C. sativum* essential (1- 40 mg/mL) and 1.6 mL of 0.1 M Tris-HCl buffer (pH= 7.4). The mixtures were shaken vigorously for 10 sec. Next, mixtures were incubated in the darkroom for 30 min. Then, the absorbance of each sample was measured at 517 nm using spectrophotometer (Shimadzu UV-1280, Japan). The antioxidant activity of the essential oil was compared to the antioxidant activity of ascorbic acid (positive control), which was tested in parallel with the essential oil in the same condition. All tests were performed in triplicate. The DPPH[•] radical scavenging is expressed as a percentage that is calculated by the following equation where Ac is the absorbance of blank (containing all reagents except the test compound) and A_s is the absorbance of samples:

Free radical scavenging (%)= $({(A_c - A_s) / A_c})^*$ 100)

The IC_{50} value is the concentration in which DPPH \degree radicals are scavenged by 50%. IC $_{50}$ of samples was calculated by interpolation and using linear regression analysis of the sample and ascorbic acid.

3. RESULTS AND DISCUSSION

3.1 HPTLC and Densitometric Analysis

3.1.1 Identification and quantification

The yield of hydro-distillation extraction for 4h was 0.16% (v/w). In this study, tolueneethylacetate (95:5 v/v) was used as mobile phase to develop HPTLC plates. The used mobile phase resulted in a reasonable separation and sharp spots. After derivatization and the densitometry scan, four spots were observed. Two spots were determined as linalool and myrcene by comparing colors and Rf values of sample and standards spots (Figs. 2,3). Table 1 shows the Rf values of sample components and standards.

The linalool content, the significant component of *C. sativum* essential oil, was assessed by densitometric analysis and establishing the standard linalool calibration curve using ImageJ software. The calibration curve of the linalool was constructed by six-point from standard concentrated 2.15-12.9 µg/spot. The linalool content in the essential oil was calculated by the regression equation obtained from the calibration curve (Table 2). The linalool content was found 60.06% (Table 1) from six repetitions of the quantitative analysis. Linalool is a monoterpene alcohol that possesses anti-inflammatory, analgesic, anxiolytic, anticancerous, antioxidative, antiseptic, antidepressive and neuroprotective, antispasmodic, antimicrobial, antifungal, expectorant, anthelminthic, diaphoretic, expectorant, anti-hyperlipidemic properties. Additionally, it can be applied as a sedative, a tonic, an immune stimulant, and a carminative. Also, it is used to treat gastrointestinal and respiratory disorders, rheumatism, menstrual disorders, wounds, and eczema [26]. K. Singh and his colleagues have reported the analysis of C.sativum seed essential oil by HPTLC. The content of the linalool was determined 52 ng/g (0.0000052%) in the essential oil [29], which shows a small amount of the linalool in comparison with the amount of linalool (60.06%) in the *C.sativum* seed essential oil from Afghanistan.

3.1.2 Validation of HPTLC method

3.1.2.1 Calibration curve and linearity

The calibration curve obtained by plotting the peak area against the concentration of standard linalool was linear in the range of 2.15-12.9 µg/spot (Fig. 4). The regression equation and correlation coefficient for linalool were y = 2137.1x + 3385.2 and 0.9919, respectively. The slop and intercept were 2137.1 ± 0.95 and 3385.2 ± 65.98, respectively (Table 2). Consequently, all data assure the linearity of the proposed HPTLC method.

3.1.2.2 Selectivity and sensitivity

The HPTLC method's selectivity was defined by conforming the sample chromatography plate and chromatogram with those of standard and comparing the Rf value (0.44) and the peak of linalool for the sample and standard. Conformity of the data was indicated the selectivity of the method.

The LOD and LOQ were 3.33 µg/spot and 10.1 µg/spot (Table 2), respectively, representing the method's sensitivity.

| Content | | Rf | Con $(*)$ ± SD |
|----------------|------------------------------------|----------------------------|-------------------|
| | Standard | Sample | |
| linalool | 0.444 | 0.441 | 60.06 ± 0.045 |
| Myrcene | 0.973 | 0.971 | |
| Total yield | | | 0.16 ± 0.47 |
| | Mobile phase front \mathbf{a} | $\mathbf b$ $\mathbf c$ | |

Table 1. Determined components of *C. sativum* **L. essential oil and concentration of linalool containing in the essential oil**

Fig. 2. The scanned image of HPTLC plate, (a) sample, (b) myrcene standard, and (c) linalool standard

Distance / arb.unit

Fig. 3. The cut of scanned image of HPTLC plate and chromatogram of the sample

Fig. 4. Calibration curve for standard linalool (n=6)

| Parameter | Linalool | |
|--------------------------------------|------------------------|--|
| Linearity-range (µg/spot) | 2.15-12.9 | |
| Regression equation (Y) | $y = 2137.1x + 3385.2$ | |
| Correlation coefficient (r) | 0.9919 | |
| $Slope \pm SD$ | 2137.1 ± 0.95 | |
| Intercept \pm SD | 3385.2 ± 65.98 | |
| Standard error of slope | 0.38 | |
| Standard error of intercept | 26.94 | |
| 95 (%) slope-confidence interval | 1843.88 - 2428.35 | |
| 95 (%) intercept confidence interval | 933.4 - 5827.22 | |
| LOD (μ g/spot) | 3.33 | |
| LOQ (μ g/spot) | 10.1 | |

Table 3. Precision studies of linalool by proposed HPTLC method (n = 6)

Standard Error

3.1.2.3 Precision

Table 3 shows the results of intra-day and interday. The results exhibited the range of RSD% (0.67- 0.71) for intra-day (repeatability) and (0.55- 0.68) for inter-day (intermediate) precision. The low values of RSD% (<1%) demonstrated the precision of the method.

3.1.2.4 Accuracy

As mentioned before, the pre-analyzed sample was spiked with the standard solution at different levels (0, 50, 100, and 150%). Table 4 shows the results of the accuracy test. The recovery range (%) was 97.1- 98.73% within an acceptable range (near 100%). Furthermore, the RSD% content for accuracy is 0.4-1.19. Accordingly, these results revealed the accuracy of the used HPTLC method is valid.

3.1.2.5 Robustness

The small changes were introduced in the mobile phase polarity to examine the robustness of the HPTLC method. Table 5 represents the robustness results. The RSD% range for the robustness experiment was 0.49- 0.73, which indicates the method's robustness.

3.1.2.6 Ruggedness

The ruggedness was performed by spotting the standard linalool with the same equipment and conditions with the different analysts. The ruggedness results were exhibited in (Table 6) which shows the RSD% range is 0.52- 0.92. The low values of RSD% have validated the ruggedness of the method.

3.2 DPPH Radical Scavenging Assay

The antioxidant activity of *C. sativum* L. seed essential oil extracted by HD was measured by

Con (mg/mL)

DPPH radical scavenging assay. In this assay, the capability of the tested essential oil was studied in terms of donating hydrogen atoms or electrons in the transformation of DPPH[•] into its reduced form DPPH-H. The antioxidant activity of the essential oil was compared with ascorbic acid (positive control). The results expressed as IC_{50} for the sample and ascorbic acid. The IC_{50} was calculated using the regression equations of sample and ascorbic acid (Fig. 5). The results of IC₅₀ of ascorbic acid and sample were reported as mean \pm SD, which are 0.02 ± 0.0004 (mg/mL) and 21.05± 0.284 (mg/mL), respectively. The IC50 for the *C. sativum* L. seed essential oil is higher than the ascorbic acid, indicating that the essential oil significantly has low antioxidant activity compared to ascorbic acid.

Table 5. Robustness studies of linalool by proposed HPTLC-method

9.5: 0.5 0,0 22673.49 ± 117.7442 0.49 0.44

| $\overline{2}$ | | | | Analyst 1 | | | 22673.49 ± 117.74 | 0.438 | 0.52 | |
|----------------|------------------------|--|-------|--|-------|-----------------------------------|--|--------------------------------|------|--|
| | | | | Analyst 2 | | | 22572.72 ± 208.1 | 0.437 | 0.92 | |
| | 50 | | | Ascorbic Acid | | a | 45 | Coriander Essential Oil | b | |
| | 40 వ్ 30 | | | | | | 40 35 వ్ 30 | | | |
| | Inhibition 20 10 | | | $y = 2696.3x + 1.6515$ $R^2 = 0.9995$ | | Ihibition 25 20 15 10 | $y = 2.1297x + 5.1797$ $R^2 = 0.9877$ | | | |
| | 0 | | 0.005 | 0.01 | 0.015 | 0.02 | 5 | | | |

Table 6. Ruggedness studies of linalool by proposed HPTLC-method Conc. (µg/spot) Analyst Peak-area ± (SD) (n = 6) Rf RSD(%)

9.6: 0.4 $+0.1$, -0.1 22756.99 ± 112.783 0.52 0.41

Fig. 5. The antioxidant activity of (a) ascorbic acid and (b) *C. sativum* **L. essential oil at different concentrations**

 $\mathbf 0$

5

10

Con (mg/mL)

15

20

4. CONCLUSION

In this study, the composition and antioxidant activity of *C. sativum* L. seed essential oil were assessed. The validity of the proposed HPTLC method proved this method is a simple, sensitive, accurate, and precise method for analyzing *C. sativum* L. essential oil. The HPTLC analysis has indicated the essential oil containing a high concentration of linalool (60.06 %). Moreover, the DPPH radical scavenging showed the seeds' essential oil has antioxidant activity. These show the medicinal and nutritional values of *C. sativum* L. seed from Afghanistan.

Afghanistan is a country rich in plant resources, including medicinal plants. Most of the Afghan population uses medicinal plants to treat their ailments due to poverty and lack of access to synthetic medicines and health care centers, especially in remote areas. Therefore, medicinal plants have particular and strategic importance for a developing and low-income country such as Afghanistan [12]. However, over the years, not enough attention has been paid to these valuable natural resources. Medicinal plants have an important place in the global economy and health system, making research on medicinal plants necessary and vital [30]. Nevertheless, there are not many reports about medicinal plants in Afghanistan, and there is a need for such research. Research on medicinal plants of Afghanistan can scientifically reveal their therapeutic and nutritional values, which can use to improve and rationalize the traditional health system. Also, the production of medicinal plants and trading in domestic and global markets pave the way for job opportunities and generate income. Therefore, medicinal plants can be considered one of the priorities and resources for self-sufficiency and improving the health and economic system of the country. In addition, economic and health policymakers should design and implement new policies to use and conserve these resources, which in the long term can significantly change Afghanistan's economy and health system [12,30,31].

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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