Future Natural Products
doi: 10.34172/fnp.2022.08 2022;8(2):35-41 http://futurenatprod.skums.ac.ir

Original Article

Formulation, microscopic and instrumental fingerprinting analysis of a multi-ingredients traditional Persian medicine product, Coriander Triphala

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Abstract

Background and aims: Today, due to the increasing use of natural and traditional medicinal products, control and standardization of herbal and traditional medicinal products is very important. As most of the traditional medicinal plants are not scientifically validated, scientific evaluation along with traditional knowledge is essential to obtain effective drugs with significant control over the quality of the product for commercial purpose. In traditional Persian medicine, various forms of medicine including one or a combination of several drugs are mentioned. Coriander Triphala widely prescribed as a purgative, gastrointestinal and mental tonic. Despite the widespread use of this drug, no significant control or standardization and documented pharmacognosy studies have been performed on it. Therefore this study is developed for this purpose.

Methods: In this study, the traditional form of Coriander Triphala was prepared based on the pharmaceutics points mentioned in traditional medicine texts and quality control and pharmacognostic studies were performed. The associated fingerprints were performed by gas chromatography–mass spectrometry (GC-MS), and then one of the main components of the product was determined by as gas chromatography-flame ionization detector (GC-FID).

Results: The spectrum of essential oils of Coriander Triphala and Coriander had 59.19% and 75.34% linalool, respectively. The microbial assay showed no undesirable results. The IR spectrum of Coriander Triphala at first day and 40 days later differed by 2%.

Conclusion: Standardization of the product using GC-FID indicates the presence of 0.172 µg linalool in 100 g the Coriander Triphala product.

Keywords: Traditional Persian medicine, Coriander Triphala, Linalool, GC-MS, GC-FID

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Received: July 4, 2022
Accepted: October 9, 2022
ePublished: November 22, 2022

Introduction

Plants, animals and microorganisms have been used since the beginning of human life as medicine to treat diseases and relieve pain (1). This productivity has gone so far as to become part of the culture and civilization of different countries (2). Today, due to the popularity of traditional medicine, many efforts have been made to prepare different formulations of traditional medicine. For this reason, quality control of traditional and plant products is very important because there is a possibility of fraud in traditional products (3). Coriander Triphala is an Persian traditional formulation (semi-solid form) that can be considered a kind of confection or electuary (4). Coriander Triphala (*Itrifil Geshnizi* in Persian) is the combination of fruits of *Terminalia chebula* Retz., *Phyllanthus emblica* L., *Terminalia bellirica* (Gaertn.) Roxb. and *Coriandrum sativum* L. along with sweet almond oil and honey. *Terminalia bellirica* (Gaertn.) Roxb. (*Balileh* in Persian), belonging to Combretaceae family, is a deciduous tree found throughout Indian forests and plains (5). The fruit is so effective in the treatment of hepatitis, bronchitis, and fever. It is used as an anthelmintic and hair tonic (6). The fruits show bronchodilatory, antiasthmatic, hypoglycemic, and spermicidal activities. Tannin, β-sitosterol, gallic acid (GA), ethyl gallate are main compounds of this plant (7). It is widely prescribed to relieve digestive problems such as stomach pain, as a food digester, bloating reliever, laxative and at the same time as an astringent, analgesic, hemorrhoid reliever, anti-nausea, anti-gastric reflux and colic pain reliever (8). *Terminalia chebula* Retz. (Combretaceae), *Halileh* in Persian, is endemic to India and Southeast Asia (9). In south east of Asia, the medicinal *Terminalia* fruit is used as a laxative and tonic agent and it is regarded as the "king of medicines". *Terminalia chebula* has been reported to have strong antioxidant capacity and high content of phenolic compounds, including GA, ellagic acid (EA), and corilagin (CG) to exhibit a variety of biological activities such as anticancer, antidiabetic, antimutagenic, antibacterial, antifungal and antiviral (10,11). It is commonly used for ulcers, gastroenteritis,

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diarrhea and cough. *Phyllanthus emblica* L., *Ameleh* in Persian, belonging to the family Euphorbiaceae, is one of the most common medicinal herbs in traditional medicine. It has been used for the hyperlipidemia, inflammation, diabetes, scurvy, cancer, heart diseases, as nutritious tonic and anti-pyretic. It is a source of tannins, vital amino acids, vitamin C and minerals (11). Coriander (*Coriandrum sativum* L., *Geshniz* in Persian) is one of the oldest spices in the world which belongs to the family Apiaceae (Umbelliferae). In traditional prescriptions introduced as a tonic, antidiarrheal and anti-vomiting agent. It is so effective for memory loss (12,13). According to Persian traditional medicine prescriptions, herbal powders of mentioned fruits should mix with honey and almond oil. Studying its essential oil and extract by gas chromatography–mass spectrometry (GC-MS), gas chromatography-flame ionization detector (GC-FID) and high-performance thin-layer chromatography (HPTLC), the standardization of this traditional medicine was investigated.

Materials and Methods

Plant materials

All needed herbs were purchased from local markets in Shiraz, Iran. They were identified in School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran and a voucher specimen of plants deposited at the Herbarium of Shiraz University of Medical Sciences.

Pre-formulation studies

The formulation was selected based on traditional prescriptions, in the authoritative books of traditional pharmacy, including *Ghrabadin Kabir*, *Ghaderi*, *Salehi*, *Azam* and *Baqaei*. The fruits of *Terminalia chebula* Retz., *Phyllanthus emblica* L., *Terminalia bellerica* (Gaertn.) Roxb. and *Coriandrum sativum* L. were pulverized separately by an electric grinder, mixed with ratio (1-1-1-3) to make the final powder. Then the powder is mixed with a very small amount of almond oil and finally, by adding honey (Twice the weight of dry ingredients) to the homogeneous amount of powder, Coriander Triphala was prepared in the form of a concoction. The final product is ready to use after 40 days which recognized as *Edrak* period.

Plants microscopic characterization

Ten grams of each plant was pulverized with a mortar and passed through a sieve with 50 mesh. The material was then poured into test tubes and chloral hydrate was poured on it. The contents of test tubes were boiled by placing on the heater stirrer. Then phloroglucinol (93 g in 25 mL EtOH) and a few drops of concentrated HCl were added. The micrographs process was performed under an electron microscope on each component of the product. The photographs of the limbs were taken in detail by a digital camera mounted on an electron microscope.

The isolation of essential oil

Essential oil of the Coriander Triphala and main component (coriander) separately were achieved by the hydro-distillation method using a Clevenger type apparatus for 4 hours. The oil was dried over anhydrous sodium sulfate and kept in amber vials at 4°C before chemical analyses.

GC-MS analysis

For analysis of the essential oils Agilent GC-MS system with HP-5MS capillary column were used. Helium was used as Carrier gas with flow rate 1 mL/min. Oven temperature was set up between 60 to 220°C. the mass range was determined between 30 to 600 m/z. In order to distinguish the essential oil ingredients, the Kováts indices were calculated via using retention times of synchronically injected normal alkanes (C9-C24).

GC-FID analysis

GC-FID was used by an Agilent GC-FID system with a HP-5 column and flame ionization detector (FID). Nitrogen, as a carrier gas, was used in the flow rate of 1 mL/min. The column temperature was programmed from 60 (0 min) to 250°C. The detector was modified 300°C. The standard solution of thymol as a reference compound was ready in pure methanol. In order to plot the calibration curve, solution of linalool (0.625, 1.5, 2.5 mg/mL) prepared by methanol and about 1 µg of each one sample injected to GC-FID for three times. Also, the essential oil yielded from Coriander Triphala was prepared (0.02 mg/mL) and injected to GC-FID three times a day in three days to determine relative standard deviation.

Attenuated total reflectance-infrared (ATR-IR) spectroscopy

According to Persian traditional treatises the final product of Coriander Triphala is usable after 40 days of preparation, a freshly prepared sample and a 40-day sample were used for infrared fingerprint. IR spectrum range for all samples was 717-3992 cm^{-1}. In the next step, the similarity of the two spectra related to the samples at two different times (the first day and after 40 days) were calculated using the similarity factor formula.

Microbial assay

For microbial evaluation, the product was sent to the food and drug control laboratory of Shiraz University of Medical Sciences to check for the presence of microorganisms.

Results

Essential oils and yield determination

The value of essential oils of Coriander Triphala and coriander (its main component), which is extracted separately by distillation with water and by Clevenger, is shown in Table 1.

Microscopic characterization

The micrograph process is the first step in fingerprint analysis of traditional products. The fruit wall or pericarp and its main parts including the exocarp, mesocarp and endocarp for coriander seeds and *Terminalia chebula* are shown in Figures 1 and 2. The microscopial characteristics *Terminalia bellirica* and *Phyllanthus emblica* are shown in Figures 3 and 4. The coriander seed was yellowish brown to brown in color, globular, oval in shape, and 2–4 mm in diameter. It had aromatic odor and spicy characteristic taste. Mericarps of the fruit were usually joined by their margins forming a cremocarp often crowned by the remains of sepals and styles.

GC-MS essential oil analysis

For determining the main compounds of Coriander Triphala about 1 µL of its essential oil were injected into GC-MS. The results of the analysis of chemical compounds and the GC-MS chromatogram of Coriander Triphala, Coriandrum sativum essential oil are demonstrate in Table 2 and Figures 5-6. According to GC-MS analysis, Linalool is the main volatile compound in the Coriander Triphala and *Coriandrum sativum* seeds. That was similar to the result of Bhuiyan et al, in determine the main compounds of leaf and seed essential oil of *Coriandrum sativum* L. from Bangladesh where the

Table 1. Essential oil extraction

Common name	Weight (g)	Essential oil (mL)	Yield (%)
Coriander Triphala	400 g	1.16 ± 0.35	0.29 ± 0.09
Coriander	800 g	1.60 ± 0.34	0.20 ± 0.04

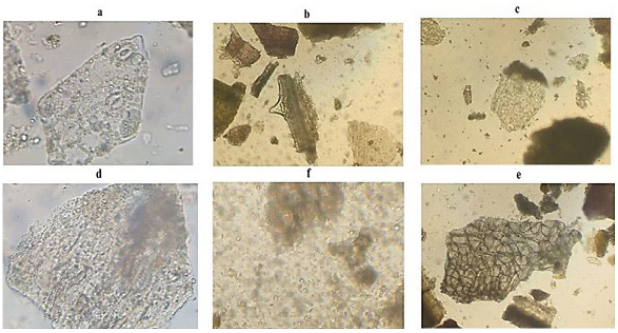

Figure 1. Microscopical characteristics of coriander seed by using hydrated chloral. (a) Epicarp epidermis. (b) The epicarp contains a transparent layer of long marginal epidermal cells with hairs. (c) Exocarp. (d) Circular fibrovascular bundle. (e) Complex network of sclereids in the endocarp. (f) Essential oils

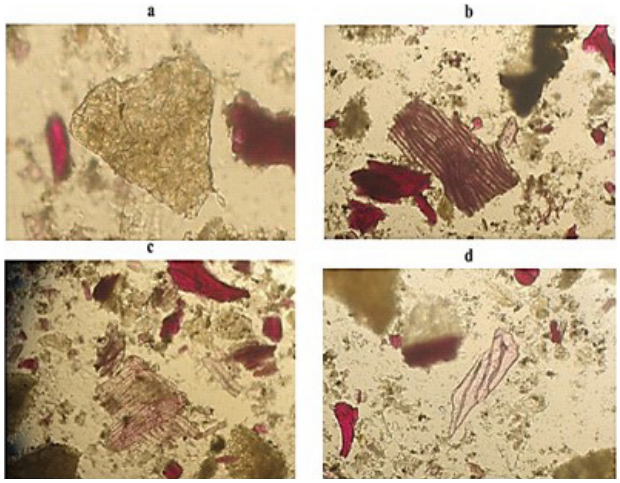

Figure 2. Microscopical characteristics of *Terminalia chebul* (d) Spotted sclereids in the endocarp

Table 2. Chemical composition of the Coriander Triphala and Coriandrum sativum seeds essential oils

Component	Coriander Triphala (%)	*Coriandrum sativum* (%)
Alpha-pinene	4.27	7.84
Beta-pinene	0.69	-
O-cymene	1.26	0.48
Gamma-terpinene	11.84	11.94
Linalool	59.19	75.34
Geranyl acetate	0.12	-
Tetradecanoic acid	0.98	-

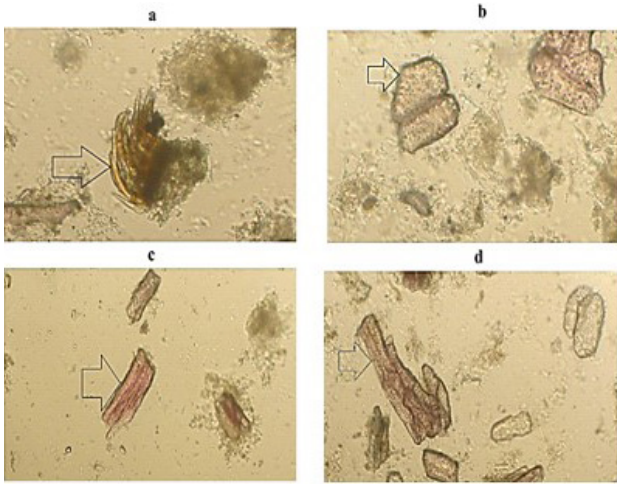

Figure 3. Microscopical characteristics of *Terminalia bellirica* by using hydrated chloral. (a) Exocarp covered with hair. (b) Spotted cells in the mesocarp. (c) Mesocarp sclerosis. (d) dotted cells

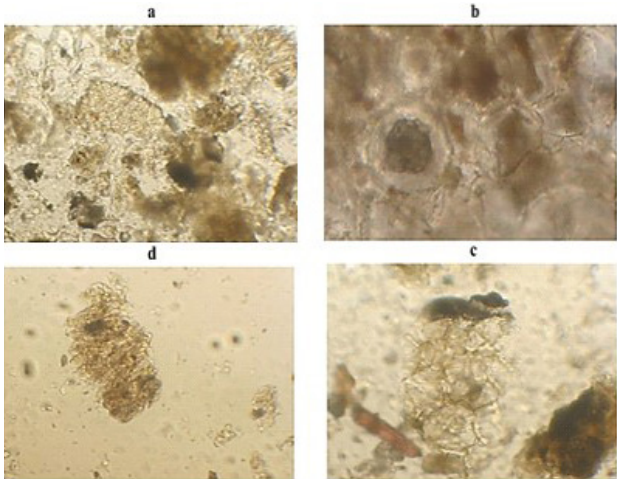

Figure 4. Microscopical characteristics of *Phyllanthus emblica* L. by using hydrated chloral. (a) Exocarp. (b) Crystalline mass in the mesocarp parenchyma. (c) Mesocarp parenchyma. (d) Testa

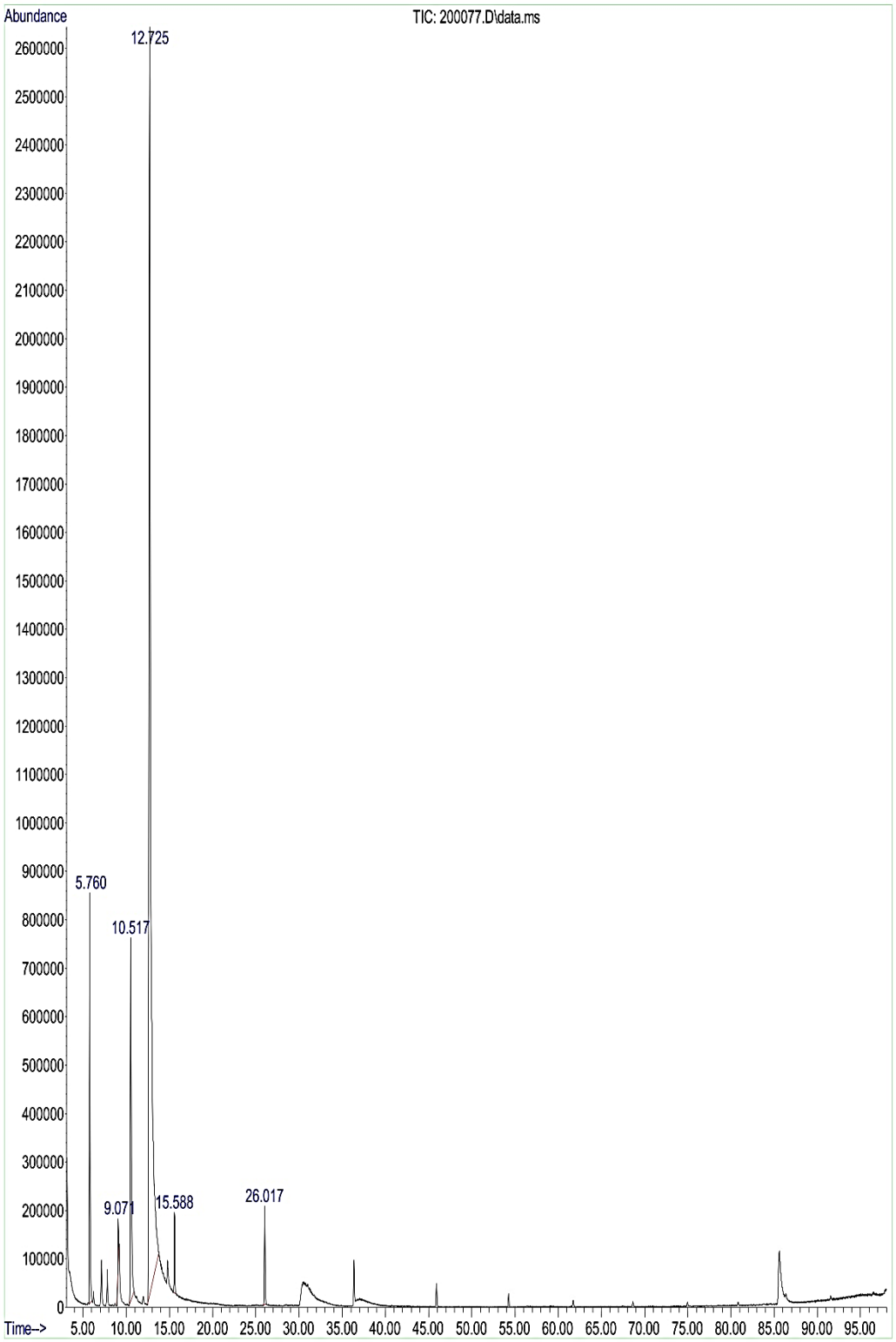

Figure 5. GC-MS chromatogram of Coriandrum sativum seeds essential oil

main compound was linalool (37.7%) (14). Or similar to the result of Msaada et al, who reported linalool as the main constituents of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity (15). Nejad Ebrahimi et al reported that almost all of the studied accessions of *Coriandrum sativum* L. contained more that 60% linalool (16).

Quantification of linalool as a major compound by GC-FID

GC-FID method was used to assess the exact amount of linalool in Coriander Triphala formulation. For this intention, the standard curve of linalool was obtained and the relevant equation was calculated according to different concentrations of the standard (Figure 7). As a

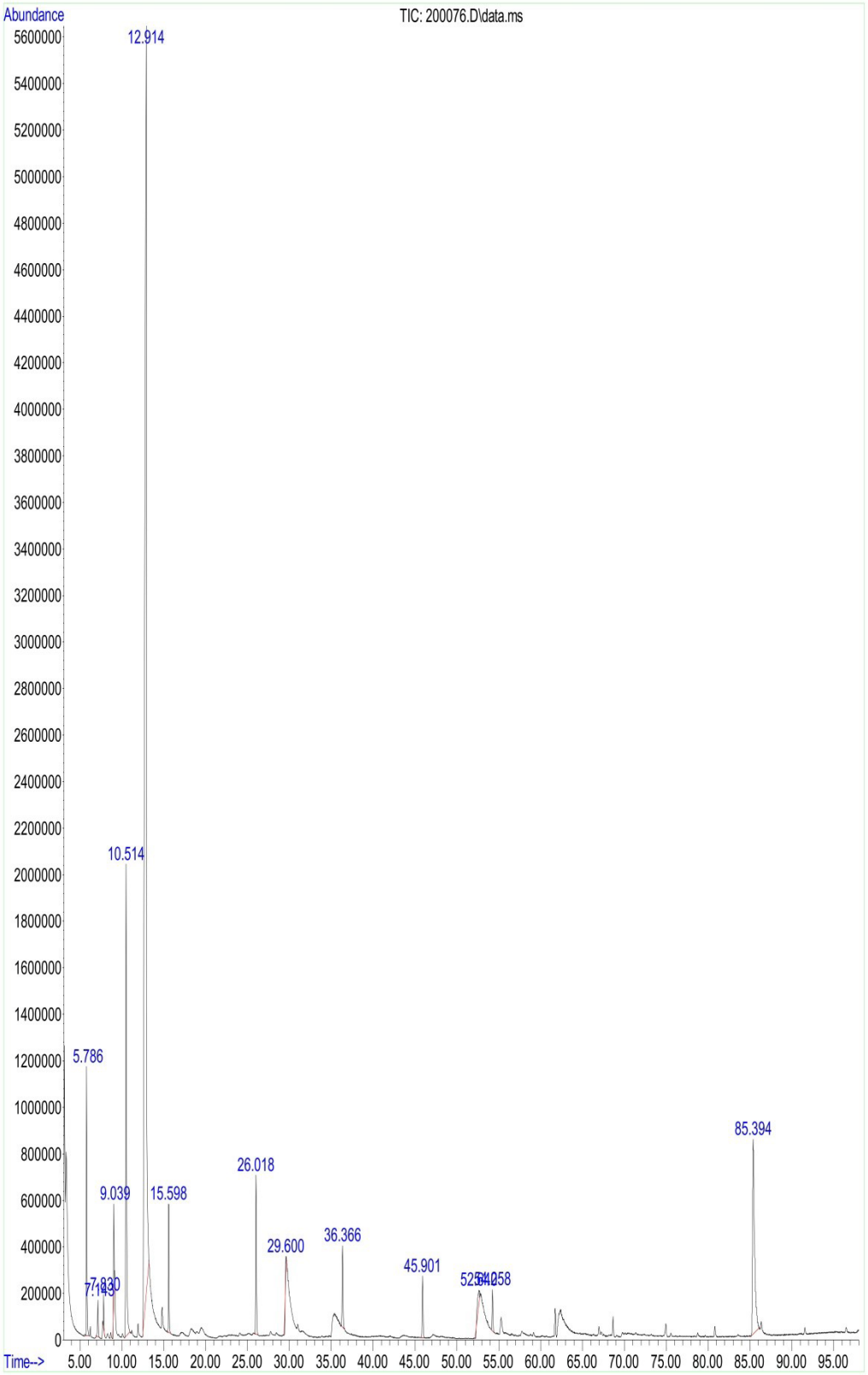

Figure 6. GC-MS chromatogram of Coriander Triphala essential oil

result, the exact amount of Linalool in a 100 g sample of Coriander Triphala is equal to 0.172 µg.

ATR-IR spectroscopy

The analysis of the IR spectrum shows that the spectra of coriander triphala at first day and 40 days later, which is related to the time of perception (*Edrak*), differed by 2%. The degree of similarity of the investigated spectra is shown in Figure 8.

Microbial assay

The total count of microorganism was fewer than

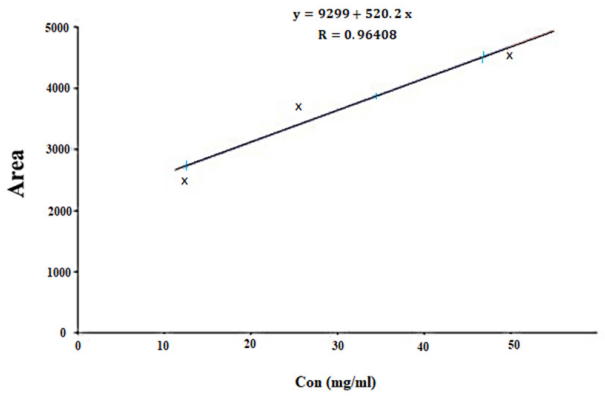

Figure 7. Standard curve of Linalool

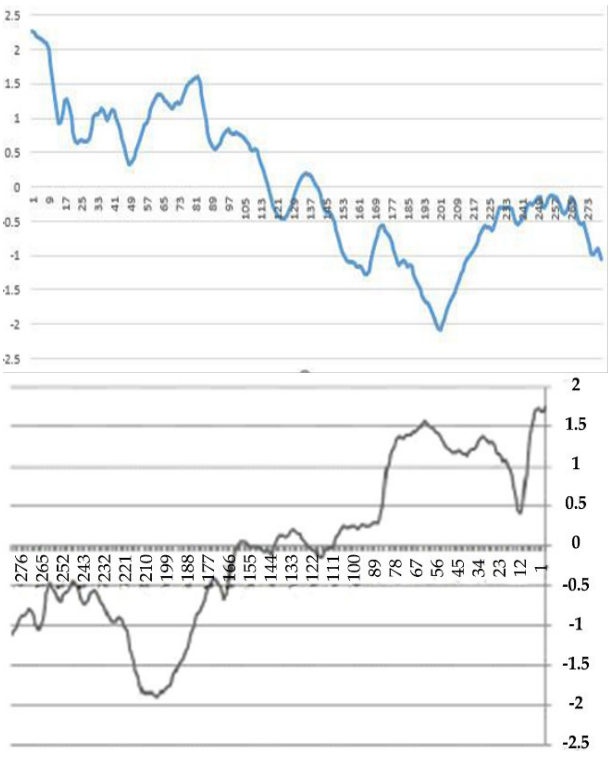

Figure 8. IR spectrum of Coriander Triphala at first (A) and 40 days (B) after production

10 000 in one gram. Tests for existence of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species, sulfite-reducing clostridia and enterococci were negative.

Discussion and Conclusion

Due to the fact that medicinal plants have a high variety, in all studies related to control and standardization, what is very important in the beginning is to identify and determine the main and correct genus and species of each of the components. Incorrect identification may lead to the manufacture of a drug other than that intended, and as a result may result in an unrelated therapeutic response or dissatisfaction with the drug. In addition, possible side effects should be considered. In this study, the medicinal form of Coriander Triphala was phytochemically evaluated. This medicinal form in all the various formulas mentioned for it and in the texts of traditional Persian medicine and pharmacy consists of 3 basic plants in the form of a concoction. These three components include *Halileh* with the scientific name of *Terminalia chebula*, *Balileh* with the scientific name of *Terminalia bellerica* and *Aemleh* with the scientific name of *Emblica officinalis*. In this study, Coriander Triphala was prepared with the mentioned ratios and in accordance with the texts of traditional medicine. In the first step, the botanical characteristics of each component were evaluated separately and in combination with each other and the identification steps were performed. This method can greatly clarify the possibility of fraud in a traditional plant product by identifying chemical components and the presence of unrelated plants. Also in this study, essential oils were extracted from both Coriander and Coriander Triphala and GC-MS spectra were prepared. The GC-MS spectrum can be used as a fingerprint to help identify coriander and Coriander Triphala. In the present study, the spectrum of coriander essential oil compounds contained 75.3% of linalool as the main ingredient, which is similar to previous studies that have introduced linalool as the main constituent of coriander essential oil. (17-19) The microbial assay showed no undesirable results. In addition, the IR spectrum of Coriander Triphala at first and 40 days later differed by 2% which determined the effect of *Edrak* time (40 days) on the preparation of medicine for use according to Traditional Persian medical books.

Author Contributions

Conceptualization: Zohreh Abolhassanzadeh, Mohammad Mehdi Zarshenas.
Methodology: Zohreh Abolhassanzadeh, Mohammad Mehdi Zarshenas, Amirhossein Sakhteman.
Validation: Zohreh Abolhassanzadeh.
Formal Analysis: Zohreh Abolhassanzadeh.
Investigation: Bahareh Rahimi.
Resources: Abolhassanzadeh.
Data Curation: Abolhassanzadeh, Mohammad Mehdi Zarshenas.
Writing—Original Draft Preparation: Zohreh Abolhassanzadeh.
Writing—Review and Editing: A Zohreh bolhassanzadeh.
Visualization: Zohreh Abolhassanzadeh.
Supervision: Zohreh Abolhassanzadeh, Mohammad Mehdi Zarshenas.
Project Administration: Zohreh Abolhassanzadeh.
Funding Acquisition: Zohreh Abolhassanzadeh.

Conflict of Interest Disclosures

The authors declare that there is no conflict of interests.

Ethical Approval

This project is based on the Pharm D. thesis of Mrs. Bahareh Rahimi, which was completed in Shiraz University of Medical Sciences with number 132 in October 2015 in supervision of Dr Zohreh Abolhassanzadeh (Ph.D)(supervisor), Dr Mohammad Mehdi Zarshenas (Ph.D)(supervisor) and Dr Amirhossein Sakhteman (Ph.D) (advisor).

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Cite this article as: Abolhassanzadeh Z, Rahimi B, Zarshenas MM, Sakhteman A. Formulation, microscopic and instrumental fingerprinting analysis of a multi-ingredients traditional persian medicine product, coriander triphala. Future Nat Prod. 2022;8(2):35-41. doi: 10.34172/fnp.2022.08.