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A Survey of the Effects of Coriander Seed Essential Oil by Solvent-Free Microwave Extraction and Ultrasonic Waves on Control of the Growth of Salmonella Typhimurium Bacteria

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ABSTRACT

The essential oils are aromatic compounds found in various organs of plants and they are called volatile or essential oil due to evaporation due to air exposure. In this study, Coriander was extracted by Solvent-Free Microwave Extraction (SFME) in three powers 450, 600 and 800 watt and ultrasonic. Then, by gas chromatography/mass spectrometry, the extracted essential oil is evaluated. The highest percentage of essence is obtained by SFME method with power 600 watt. The results show that to have essential oil enriched with oxygen compound with anti-bacteria properties, SFME method with power 600 watt is suitable. One of the advantages of this method is the lack of need to high temperature and high organic solvent. Then, by the extract of this method, the anti-bacterial properties of Coriander on the growth of Salmonella typhimurium bacteria are evaluated and this bacterium is one of the important pathogenic factors in medicine and veterinarian. The results showed that Coriander seed essential oil in low concentration (30 ppm) has bacteriostatic effects. By increasing the concentration, this effect is increased as in concentration 100ppm, the essential oil has bactericidal effect. Thus, it can be said Coriander seed essential oil with anti-microbial components considerably can be used in controlling the growth of Salmonella bacteria.

KEYWORDS: Coriander, Solvent-Free Microwave Extraction (SFME), Ultrasonic, Gas chromatography/mass spectrometry.

INTRODUCTION

The essential oils are aromatic compounds found in various organs of plants and they are called volatile or essential oil due to evaporation due to air exposure.

Essential oils are a class of herbal oils composed of the mixture of volatile organic chemical compounds and they generate good smell or taste in plant. Based on the type of plant species, essential oils can be found in many plants. The most important species with volatile oil include pine, Laurus nobilis, sour orange, Myrtus, apiaceous, menthe spicata and Chicory. (Gurdeep, R. Chatwal., 1991)

History and source of Coriander

Coriander is one of valuable medicinal herbs applied in pharmacology industries of advanced countries and is cultivated in various regions and Iran. Coriander seed is found in Egypt in Tutankhamun tomb and other ancient Egypt tombs. The important point is that in ancient Egypt, various cultivars of Coriander from Asia are referred. The source of this plant is not clear and there is not much information about its wild species. Some researchers consider Coriander as a wild plant. Linnaeus reported as long ago as 1780 that coriander also occurs as a weed in cereals. In China, coriander is mentioned as a vegetable in a book on agriculture from the 5th century. (R. H., Thomson, 1993)

Coriander fruit falls in harvest in field and is week in the next cultivation. The lists of centers of origin of cultivated plants, Vavilov mentioned coriander for central Asia and Ethiopia in each of regions, various types of Coriander are found. Coriander cultivation has started regularly since 16th century and it has been cultivated in various regions for medicine consumption. The fruit and growing part of Coriander has essential oil. This plant in most of pharmacopoeia is introduced as medicinal herb. The specific smell and taste of Coriander is due to its essential oil. This essential oil is used mostly in food, health, cosmetic, soft-drink, chocolate and in pharmacology industries. The annual production of Coriander in the world is more than 220 thousands of Ton and about 60% are produced in Russia. This country extracts annually 1000 ton of Coriander essential oil via steam distillation. The most important countries producing Coriander are Russia, India, Italy, Bulgaria, Spain, Morocco, Romania, Netherland, Pakistan, England and US. (Harborne, J. B., 1988)

Active ingredients in Coriander seed

The aromatic oil in coriander is a digestive stimulant. The oil contains linalool and other important terpenoids. Other active compounds in coriander include flavonoids, phenolic acids and mucilage (a soluble fiber). Coriander also contains a number of substances with mild anti-bacterial activity. Preliminary reports suggest that both cilantro and the coriander seeds contain dodecanal, a natural antibiotic that protects against food-borne illnesses caused by Salmonella. Coriander seeds also contain phthalides and polyacetylenes. These phytochemcials, commonly found in plants belonging to the parsley family, are protective against cancer. They also contain small amounts of coumarins, substances that

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possess blood thinning properties. Coriander also contains antioxidant and anti-inflammatory compounds. (Heegauer, R., 1991)

Anti-microbial properties

Coriander consumption is recommended in various infectious diseases as (Typhoid fever and various diseases with general source of bacillus and seed fever). Its anti-microbial and anti-fungus effects against Bacillus anthracis, Bacillus mesentericus, Proteus vulgaris, Escherichia coli, Staphylococcus, aureus, hemolytic streptococci, Pseudomonas aeruginosa and Candida albicans. (Guenther, E., 1975)

Safe application of this plant: As a herb, coriander is entirely safe, has no side effects, no contraindications, and can be safely used during pregnancy. Ground coriander is apt to lose its flavor quickly so that one should store it in an opaque airtight container in a cool, dark place. (Weiss, E. A., 1977)

Anti-bacterial mechanism

Based on the number of chemical compounds in plants essential oil, we cannot consider a unified mechanism for their anti-bacterial effects and they have various purposes in cell. These mechanisms don't act separately and some of them are affected by others. One of the important features of essential oils and their constituents is their hydrophobia nature as these materials can infiltrate into lipids of bacterium cell membrane and mitochondrial and their structure can be disturbed and it leads to high infiltration. This leads to the leakage of ions and other cellular contents and the exit of limited values of these materials is tolerable for bacterium but its biological capability is affected and the exit of extensive amounts of cellular contents or ions and vital molecular exit lead to the death of cell. (Bhattacharjee, S. K., 2000)

Generally, the higher phenolic materials in essential oil, the higher their antibacterial properties against food pathogens. These ingredients include carvacrol, eugenol and thymol. The mechanism of the effect of these ingredients like other phenolic compounds includes the disturbance in cytoplasmic membrane, imbalance of proton movement and electric flow and agglutination of cellular contents. Chemical structure of essential oil affects its mechanism and the importance of the presence of hydroxyl group in phenol compound as carvacrol, and thymol is supported. The relative situation of hydroxyl group in phenolic ring has no effect on its anti-bacterial effect. For example, the impact of thymol on bacillus cerus, Staphylococcus, aureus and Pseudomonas aeruginosa is similar to the impact of carvacrol. In a study, it is shown that carvacrol and thymol has different effects on gram positive and negative types. Essential oil composition is effective on existing proteins in cellular membrane. (Tyler, V. E.; Brady, L. R., 1987)

Bacteria properties

Bar form bacterium, gram negative with different length and its species except Salmonella gallinarum and move by surrounding flagella, grow in simple cultivation medium but they cannot perform lactose and sucrose fermentation. Glucose and Mannose are fermented and sometimes generate acid and gas. Normally, they generate sulfurized hydrogen and they continue in long freezing periods. Some chemical materials as green, sodium tetrathionate and Sodium deoxycholate are strong but coliforms are sensitive and coliforms are used in most cultivation medium for separation of Salmonella and their separation. From structural points, an antigen with antigen O (more than 60 types of antigens) and antigen H are in one or two different phases. Some types of Salmonella have capsule (antigen K) called V_i. This antigen interferes in agglutination reaction with O antigen and is associated with organism invasion power. The division is based on the type of O, H antigens and applies Kauffmann-White system. (Buugh, P. J., 1994)

Essential oil extraction method

Extraction method by solvent-free microwave

By Solvent-Free Microwave Extraction method, at first 100gr of Coriander seed are grinded, then by submersion method, they are soaked for one hour in water. This stage is performed due to the increase of initial humidity of dry plant and it is necessary. After one hour, extra water is rinsed and the seed is kept in microwave. This type of heating with microwave oven is with distillation at atmosphere pressure. Heating the water inside cell of plant leads to its inflammation and blasting of glands and supporting tissues of essential oil and the essential oil with intracellular water is evaporated and then the steam moves to a condenser outside of the oven box and accumulates there. The essential oil is collected in a flask and extra water is returned into extraction container to keep the humidity of plant materials. During process, the temperature is controlled via a thermocouple being put directly inside reactor. The total extraction process of essential oil in 30 min is performed to 450, 600 and 800 watt microwave. Then, the required essential is rinsed by dehydrated sulfate sodium and is kept in refrigerator to the analysis time and the essential oil yield is determined. An image of microwave system is shown in Figure 1-1. (Hubschmann, H. J., 2000)

Fig. 1. A view of applied microwave system

Essential oil extraction by ultrasonic energy

Power 100watt and frequency 40kHertz are applied for extraction by ultrasonic (Figure 1-2). Extraction by Hexane solvent is performed as followings: At first, 30gr grinded seed are poured into container 500mL and about 75mL hexane solvent are added and a rotary is used and kept in ultrasonic system and is kept for 22min in ultrasonic waves at 60℃. The grinded seed is separated from solution by a filter paper and the solution passes the filter and is kept in rotary to be concentrated and solvent can be separated from essential oil and this is performed to equality of solvent of rotary with solvent amount in grinded seed and then essential oil yield is defined and the required essential oil is injected to GC/Mass device. (Atkinson, D. and J. R. Porter, 1996)

Fig. 2. A view of ultrasonic system in this study

Determining essential oil yield

By dry plant weight in extraction and the weight of essential oil, the essential oil yield is obtained as weight /weight ratio (w/w).

Identification of essential oil constituents

After preparation of essential oil by GC/Mass, constituent of essential oil are identified qualitatively and quantitatively. The identification of compounds is performed by some parameters as time, inhibition index, the study of mass spectrums and the comparison of these spectrums with standard compositions and existing information in computer library of GC/Mass. The relative percent of each of compounds is obtained based on its sub-curve level in GC chromatogram by Area normalization method and ignoring Response factors. (Wheaton, E.E. 1994)

Determining the anti-bacterial impact of Coriander seed essential oil

Test organism

Lyophilized cultivation of Salmonella typhimurium bacteria provided from scientific and industrial studies of Iran is applied for this process. At first, Lyophilized cultivation is cultivated twice in nutrient broth medium (NB) at 37℃ for 18 hours, in the second cultivation, it is mixed with sterile glycerin as 5 to 1 and is kept equally in sterile Eppendorf microcentrifuge tubes at -20℃.(Chmielewski, F.M., A., Müller, and E., Bruns, 2003)

Providing bacteria inoculation

The inoculation of Salmonella typhimurium bacteria is performed by transferring bacteria from Eppendorf microcentrifuge to NB and keeping it for 18 hours at 37℃, second culture is provided from this first 18 hours culture in nutrition broth medium (NB)(for 18 hours at 37℃).

Then, Cuvett tubes with 5mL sterile nutrient broth are provided. Various amounts of nutrient broth cultivation of second 18 hours were carried on various Cuvett tubes, of contents of these tubes, bacterium count is performed by Piur

Plate method and finally Cuvett tube with 107 bacterium in each mL is defined and in each test, by defining optical absorption equal to about 107 bacterium in each mL (then by cultivation of Pour Plaste, it is proved), Cuvett tube with 107 bacterium in each mL is also defined. (Bradley, N.L., Leopold, 1999)

Providing nutrient broth medium (NB) by required concentrations of essential oil

To provide 100mL basic broth medium, 20gr NB cultivation medium is dissolved in 90mL distilled water in an Erlenmeyer Flask with mild heat, then 5% of DMSO solution (as Emulsifiers) are added and final volume is increased to 100mL by distilled water and is used in autoclave (121℃ for 15min) for sterilization. Finally, various amounts of essential oil are computed to provide the medium with required concentrations as zero, 30, 60, 125, 250, 500, 1000ppm. (Shibamoto, T., 1987)

Inoculation of nutrient broth and determining anti-bacterial impact

All the required dilutions with various amounts of essential oil as 350macroliter are poured into micro plate wells of Bioscreenc. Various dilutions of Salmonella typhimurium bacteria are added to all wells as 50 macroliter. It can be said 2 replications are considered for all concentrations.

Then, each micro plate is put at temperatures 35℃ in Bioscreenc and after controlling temperature and wave length of device for 20 hours in each 10min, the optical absorption of all wells is read and it is increased by increasing bacterium growth. After the above test, bacteria count is performed of all concentrations. Totally, all the results are analyzed by SPSS 16 software.

The properties of the essential oil by SFME extraction method

For optimization of the impact of microwave power, three powers 450, 600 and 800watt are used for essential oil extraction. The list of constituents of essential oil is shown in the following Tables with inhibition index (RTm or inhibition time) and relevant percentage is also mentioned. As shown, the main compounds qualitatively are not changed in power 450 and 600 watt but quantitatively, the percent of oxygen compound at power 600watt is higher than power 450. Power 800 destroys essential compound and as shown, the percent and type of compound in power 800 watt is different from two previous powers. At power 800watt, oxygen compounds are not main com pounds. One of the advantages of SFME method is the lack of need to high organic solvents. thus, essential oil cost is lowered and less damage is imposed on environment. It is a green and environment friendly method. Another important feature of this method is the lack of need to high heat for extraction. High temperature has adverse effect on essential oil compounds. The highest percent of essential oil at power 600 is regarding the following compounds:
Dodecan-1al-2‹(10.21) γ-Terpinene ‹(6.21) α-Pinene‹(14.80) Borneol‹(13.12) Carene‹(13.69) Camphene (4.12) Limonene‹(16.5) Nerol ‹(12.60).

Table 1. The identified compounds and their percent in Coriander essential oil (Extraction method: SFME and power 450watt)

% Of total	RTm	The composition	No.
8.24	10.670	α-Pinene	**1**
2.70	11.812	β-Pinene	**2**
3.18	13.940	I-Limonene	**3**
7.90	15.540	γ-terpinene	**4**
30.122	19.770	Camphene	**5**
0.8	20.168	bicyclo[2,2,1]heptan-2-one,1,7,7	**6**
12.01	20.718	Borneol	**7**
0.408	21.518	Decanal	**8**
1.10	22.123	β-citronellol	**9**
10.22	22.780	Nerol	**10**
0.24	23.422	1-Dodecanol	**11**
0.37	24.115	Undecanal	**12**
0.2	24.826	1(7),5,8-0-methatriene	**13**
12.6	26.780	3-carene	**14**
0.43	27.414	Dodecanal	**15**
8.40	29.280	2-Dodecan-1al	**16**
0.22	36.120	9-Octadecene,(E)	**17**
0.34	44.720	Octadecane	**18**
0.52	53.378	Hexzadecane	**19**

Table2. The identified compounds and their percent in Coriander essential oil (Extraction method: SFME and power 600watt)

% Of total	RTm	The composition	No.
10.10	10.420	α-Pinene	1
2.78	11.780	β-Pinene	2
4.12	13.820	I-Limonene	3
6.21	15.120	γ-terpinene	4
13.69	19.320	Camphene	5
14.80	20.590	Borneol	6
0.8	21.617	Decanal	7
1.80	22.320	Beta citronellol	8
16.50	22.817	Nerol	9
0.36	23.370	1-Dodecanol	10
1.02	24.120	Undecanal	11
0.32	24.850	1(7),5,8-0-methatriene	12
13.12	26.650	3-carene	13
0.78	27.410	Dodecanal	14
12.60	29.150	Dodecan-1al-2	15
0.1	36.10	9-Octadecene,(E)	16
0.25	44.780	Octadecane	17
0.65	53.390	Hexzadecane	18

Essential oil yield = (essential oil Coriander weight/weight of) × 100 = (0.36×100) / 100= 0.36

Table 3. The identified compounds and their percent in Coriander essential oil (Extraction method: SFME and power 800watt)

% Of total	RTm	The composition	No.
20.92	10.322	α-Pinene	1
3.50	11.720	β-Pinene	2
8.20	13.960	I-Limonene	3
14.80	15.019	γ-terpinene	4
3.29	19.322	Camphene	5
2.22	20.790	Borneol	6
3.50	22.450	β-citronellol	7
2.80	22.990	Nerol	8
2.45	23.350	1-Dodecanol	9
2.60	24.180	Undecanal	10
2.50	24.401	1(7),5,8-0-methatriene	11
25.10	26.580	3-carene	12
2.50	27.401	Dodecanal	13
2.50	29.103	Dodecan-1al-2	14
1.1	35.316	9-Octadecene,(E)	15
1.2	44.716	Octadecane	16
0.82	53.401	Hexzadecane	17

0.10= 100) / 100×0.10 = (100) × essential oil Coriander weight/weight of = (Essential oil yield

Properties of obtained essential oil by extraction method by ultrasonic waves

Ultrasonic waves of hexane solvent are used in extraction. Table 1-4 shows the list of constituents of essential oil with inhibition index and percent. The main extracted matter with this solvent is Cyclofenchene with 77%.

Table 4. The identified compounds and their percent in Coriander essential oil (Extraction method: ultrasonic and hexane solvent)

% Of total	RTm	The composition	No.
0.26	8.565	p-xylence	1
4.67	10.361	α-Pinene	2
0.76	11.708	β-Pinene	3
18.68	13.744	I-limonene	4
3.43	14.889	Acetophenone	5
59.65	16.922	3-carene	6
3.82	26.316	careen	7
0.78	28.988	2-Dodecan-1-al	8
4.00	55.783	Octadecan	9
3.95	60.412	Octadecan	10

Essential oil yield = (essential oil Coriander weight/weight of) × 100 = (0.21×100) / 100= 0.21

One of the advantages of extraction method by ultrasonic waves is the simplicity and cheapness of the method. Also, extraction yield is high and extraction is not performed at high temperature and this leads to avoiding thermal destruction. This method is performed by any solvent. However, one of the drawbacks of this method is requiring filtration and separating the extract after extraction. Also, organic solvent is used. Although, this method is selected by specific solvents but this method is considered as non-selective entirely.

The results of anti-bacterial effect test of Coriander seed essential oil

After performing test by Bioscreenc as shown in the previous chapter, the anti-bacterial impact of Coriander seed essential oil is shown well in Figure 1-3.

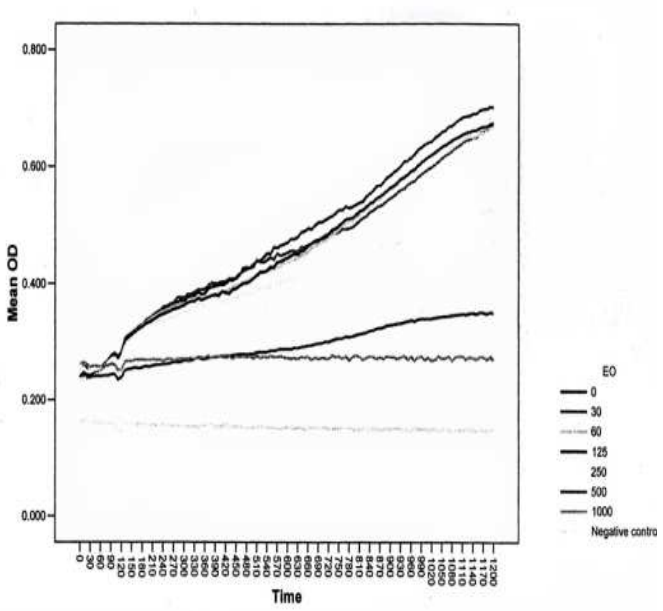

Fig. 3. The anti-bacterial effect of Coriander seed essential oil on growth of Salmonella typhimurium bacteria

As shown in the chart and based on the statistical test of variance analysis between zero concentrations and other concentrations, there is a significant difference and it shows that essential oil even at very low concentrations can reduced bacterial growth.

There is no significant difference between concentrations 30, 60 and 125 but there is a significant difference between the above concentrations and concentration 250 ($P<0.05$) and there is no significant difference between concentrations 500 and 1000. It means that in both concentrations, the inhibition effect of essential oil is similar.

In the present study, after performing the test, there is no growth of all cultured concentrations alone at concentration 1000 and all bacteria are killed in the above concentration.

Conclusion

Essential oil yield to seed dry weight for various extraction methods is shown in Table 1-5. As shown, essential oil yield in SFME is higher than other methods. Also, at power 600 microwave, the highest essential oil yield is obtained. This is one of the great advantages of this method and it is used at industrial scale. By this method and without the need to high temperature and with low consumption of organic solvent, the highest essential oil percent is extracted. Generally, by various methods regarding extracted essential oil percent we can say that:

(power 800) SFME < ultrasonic < power 450) SFME <(power 600) SFME

Table 5. The percent yield of essential oil by various extraction methods

Extraction efficiency (% w / w)	The extraction method		No.
0.32	450 W	SFME	**1**
0.36	600W		
0.10	800W		
0.21	Ultrasonic		**2**

The most important compounds obtained by Coriander essential oil by various methods are summarized in Table 1-6.

As shown, in SFME method, the compounds with high percent are oxygen compound (Nerol، Borneol and Dodecan-1al). The extracted compounds depend fully upon applied extraction method and temperature. To extract the compounds with hydrogen and carbon, other methods are suitable.

Table 6. A summary of most important extracted compounds by various methods

The extracted compounds	The extraction method		No.
Camphene (30.12), Carene (12.6), Borneol (12.01), α-Pinene (8.24), 2-Dodecan-1al (8.40), Nerol (10.22)	450	SFME	**1**
Camphene (13.69), Carene (13.12), Borneol (14.80), α-Pinene (10.10), 2-Dodecan-1al (12.60), Nerol (16.5)	600		
β-Pinene (3.50), Carene (25.10), α-Pinene (20.92), γ-Terpinene (14.80), Limonene (8.20)	800		
Carene (3.66), γ-Terpinene (3.31), Limonene (7.43), Cyclofenchene (76.53)	Ultrasonic		**2**

In two methods, most of the compounds being extracted have oxygen. The extraction of these compounds by SFME method is higher than other methods. Oxygen compound in terms of odor and smelling and anti-microbial and anti-oxidant properties have high value compared to other compounds. High value of oxygen compounds in SFME method is dedicated to rapid heating of polar compounds by microwave waves and reduction of essential oil extraction time and applied less water in this method. The mentioned factors reduce the degradation of oxygen compounds by thermal and hydrolytic reactions and the percent of main oxygen compounds is increased and the percent of other minor compounds is reduced. Generally, the advantages of using microwave energy as a non-contract thermal source to extract essential oil of herbal materials are including high effective heat, low thermal loss, selective heating, reduction of tools size, rapid response to thermal control processes, increasing extraction yield, elimination of some stages of process and reduction of consumed energy and time, reduction of poison risk of solvent residual and reduction of production costs. Microwave improves releasing volatile compounds of herbal matrices. Microwave waves break the glands wall rapidly and extraction yield can be increased and extraction time is reduced. Thus, extraction yield is increased to power 600watt and then it is reduced. This reduction is due to the destruction of essential oil constituents at power higher than 600watt and power 600watt is selected as optimal point of essential oil extraction by SFME method. The result showed that Coriander seed essential at low concentration (30 ppm) showed anti-bacterial properties. Also, at concentration 100ppm, no growth is observed, it means that at this concentration, all bacteria are killed and we can say Coriander seed essential oil is the best method of controlling Salmonella bacteria.

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