

## Isolation, Identification and Biological Assay of coriander oil from *coriandrum sativum* as antibiotic

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Received: 11/12/2003 Accepted: 2/11/2004

### Abstract

This study is designed to extract and identify the essential coriander oil from *Coriandrum sativum*. The Antimicrobial activity of Coriander oil was studied in various microorganisms (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) using inhibition zone method (Aromatogram). The Extraction process is carried out by steam distillation. Optimum organic extractant determined. The collected oil is identified via Thin Layer Chromatography (TLC) using a mixture of Ethylacetate : Toluene (1:9) as a mobile phase. The MIC for each microbes were estimated.

### الخلاصة

تم أعداد هذا البحث لأستخلاص وتشخيص ثم دراسة الفعالية المضادة للأحياء المجهرية لأحد أهم الزيوت النباتية الطبية من نبات الكزبرة وهو ما يعرف بزيت الكورياندر والمستخدم في العديد من المستحضرات الصيدلانية. درست الفعالية المضادة للأحياء المجهرية على عينات متعددة من البكتريا والخمائر شملت ما يلي *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* و *Proteus vulgaris* وباستخدام طريقة تثبيط المنطقة. تمت عملية الاستخلاص باستخدام التقطير البخار وباستخدام مذيب عضوي مناسب. كما تم خلال البحث تحديد المستخلص العضوي الأمثل لعملية الاستخلاص. شخص الزيت المستخلص من نبات الكزبرة بواسطة كروماتوغرافيا الطبقة الرقيقة حيث استخدم مزيج من الايثيل استيت : تولوين بنسبة 1:9 كطور متحرك. كذلك تم تحديد قيمة MIC لكل نوع من الأحياء المجهرية.

### Introduction

*Coriandrum sativum* (Umbelliferae-Apiaceae) (coriander) (common name in Arabic, kuzbura or kuzbara) has many different uses and these are based on the different parts of the plant. Coriander has been used in medicine for thousands of years [1]. The parts of the coriander used are its leaves, seeds and oil. The fresh leaves and ripe seeds have quite different aromas and uses. Both the leaves and seeds are rich in volatile oils that act mainly on the digestive system, stimulating the appetite and relieving irritation. They also act as an expectorant. The oil is fungicidal and bactericidal. The, leaves are widely used to flavor food, especially in the Middle East, and southeast Asia. The seeds are

also an ingredient of curries and pickling spices, dishes a la grecque, and bakery products. Medicinally, coriander is used internally for minor digestive problems, and externally for hemorrhoids and painful joints (seeds). Seeds reduce griping in laxative preparations based on *Rheum officinale* and *Cassia angustifolia*. The oil adds to the flavor of gin, vermouth and Chartreuse, and is also prized in perfumery [2]. General references to coriander's medical uses are also found in classical Greek and Latin literature [3], and instructions to cultivate coriander are contained in the German emperor Charlemagne's decree 'Capitulare de villis' in 812 (Göck 1977). The coriander fruits are believed to aid digestion. Many other fruits of

umbelliferous plants have been used in medicine since antiquity [4] as they also affect the digestive system and some act as an aphrodisiac. Some of these, such as hemlock (*Conium maculatum* L.), are poisonous. Coriander is also used externally to treat ulcers and rheumatism; The fruits need to be soaked in wine or in vinegar overnight before being re-dried, in order to remove chemical compounds contained in the fresh fruits, which cause dizziness) [2].

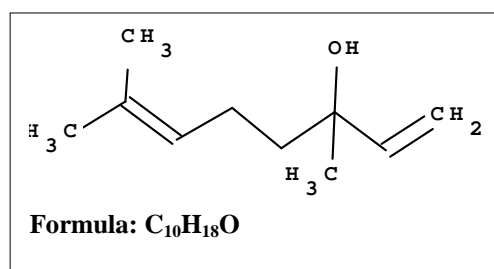


**Figure (1): Coriandrum Sativum  
(green parts and fruits)**

Fruits thus treated were used for medicinal purposes, and also to treat halitosis. Today, the plant is still sometimes used for these purposes in folk medicine. The medical uses of coriander in the modern era are described by Cicin [5]. In India, the fruits are considered carminative, diuretic, tonic, stomachic, antibilious, refrigerant. The first factory for the steam distillation of the essential oil of coriander was built in Russia in 1885 in the Vorone district [2]. In this area, the introduction of coriander as a field crop began as early as 1830, and it remains the principal producer of coriander for this purpose. The oil is obtained by steam distillation of the crushed fruits and a continuous and completely automated processing technique has been developed [6]

Recently, the essential oil has also been processed by liquid carbon dioxide extraction. The extracted essential oil is used in the flavouring of a number of food products and in soap manufacture. It is principally used as a flavouring agent in the liquor, cocoa and chocolate industries.. coriander oil has the advantage of being more stable and of retaining its agreeable odour longer than any other oil of its class. Decylaldehyde (yield 0.1% of the weight of coriander oil), obtained by treating the oil with bisulphite, was reported to be useful for perfumery purposes. The commercial oil is extensively adulterated with sweet orange oil, cedar-wood oil, turpentine and anethole or aniseed oil [7]. The main component, linalool, is used as a base for further technical processing.

Today, oleochemically synthesized linalool is usually used in the non-food sector, as it is cheaper at present. The demand for essential oils is rising in Western countries, and the full potential of this use of coriander has not yet been recognized [8]. Some countries improved the cultivation and research of medicinal plants including chemical constituents, their therapeutic application in addition to morphology and taxonomy. The essential oil obtained from the dried ripe fruit of *Coriandrum sativum* known as coriander oil, pale yellow liquid, almost insoluble in water soluble in three volumes of 70% Ethanol very soluble in chloroform, ether and glacial acetic acid. Coriander oil chiefly contains about 70% Linalool oil the active ingredient used in pharmaceutical industries as carminative and aromatics, chemical name 3,7-dimethyl-1,6-octadien-3-ol, additional name 2,6-dimethyl-2,7-octadien-6-ol, Structure and Molecular formula shown in figure (2). Linalool oil also occurs in oil of Ceylon, cinnamons, sassafras, orange flower, bergamot Artemisia and balchanorum.



**Figure (2): Chemical structure and  
Molecular formula for Linalool oil**

Many peoples used coriander oil (linalool oil) as oral wash when oral cavity is inflamed, and their was no paper in the literatures shows how can

isolate and use of coriander oil from dried fruits in medicine as pharmaceuticals, therefore, in the presence work we are established a novel method for isolate and identify the linalool oil from *Coriandrum sativum* and to study the biological activity of this oil as an antimicrobial agent.

### Materials and Method

- i. **Chemicals:** Ethanol absolute 99.9% (BDH), Ethanol 98% (BDH), Ethylacetate (Fluka), Toluene (Fluka), Methanol 98% (BDH), chloroform (Merck, Darmstadt), Hexane (AnalaR), sodium sulphate (BDH), KIESEL GEL (DF-5) 5% CaSO<sub>4</sub>.
- ii. **Sterilization:** (a): Cultures media were sterilized by autocleaving at 121°C, 15 pound/In<sup>2</sup> for 15 minutes.
- iii. **Test organisms and inoculation preparation**  
Organisms were obtained from cultures collected from mouth, wound swabs and urine of patients referred to central health Laboratories and Laboratories of Al-Yarmook hospital. Isolates were as follows; *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, *Candida albicans* and *Escherichia coli* isolated from mouth swabs, *Pseudomonas aeruginosa* was isolated from wounds, *Proteus vulgaris* was isolated from urine samples. All isolates were maintained on blood agar overnight, cultures were prepared by inoculating 2-3 colonies into 2-3 mL nutrient broth and incubating overnight at 37°C with shaking for the agar dilution assay, overnight culture were diluted in nutrient broth to approximately 10<sup>8</sup> cell/mL according to MacFerland tubes [9].
- iv. **Extraction of Coriander oil:** *Coriandrum sativum* ripe dried fruits were broken into small pieces under sterile conditions, 20 g of powdered fruits were dissolved with 150 mL of ethanol, ethyl acetate, methanol and chloroform solvents then extracted by using steam distillation apparatus for 30 minutes. The determination of Coriander oil was carried out by the steam distillation apparatus. The distillate is collected in a graduated separatory funnel. The water-free mixture of volatile oil was recovered as follows: A 0.1 ml of 0.1% w/v solution of *sodium fluorescein* (to colored the aqueous layer) and 0.5 ml of water was introduced to the distillate and leave to stand for 5 minutes then water separated from the lower tab of separatory funnel. The volume of Coriander oil measured directly by adding xylene to take up the Coriander oil. The content of oil is expressed as a percentage v/w (oil volume/weight of *Coriandrum sativum* powder). Optimum solvent extractant was determined, see Table (1). The extract thus obtained was injected into dark sterilized container.
- v. **Identification of linalool oil:** Developing thin-layer chromatography using KIESEL GEL (DF-5) 5% CaSO<sub>4</sub> for thin layer chromatography, as the coating substance.  
**Test solution.** 0.50 mL of coriander oil was shaken with 5.0 ml of hexane for 2-3 min and filter over 2 g of anhydrous sodium sulphate  
**Reference solution.** 15 µl of linalol and 25 µl of olive oil was dissolved in 5.0 ml of hexane immediately before use.  
Apply to the plate as bands 20 µl of the test solution and 10 µl of the reference solution. The chromatogram was develop over a path of 10 cm using a mixture of 5 volumes of ethyl acetate and 95 volumes of toluene. The plate was dried in air and develop again in the same conditions. The plate was sprayed with anisaldehyde solution and examined in daylight while heating at 100°C to 105°C for 5 min to 10 min. The chromatogram obtained with the reference solution shows in the lower half a violet to greyish-violet zone (linalol) and in the upper half a bluish-violet zone (triglycerides). The chromatogram obtained with the test solution shows zones similar in position and colour to the zones in the chromatogram obtained with the reference solution. Several violet-grey to brownish zones, including the zone corresponding to geraniol, are between the starting point and the zone due to linalol in the chromatogram obtained with the reference solution. It may also show several faint violet-grey zones between the zone due to triglycerides and that due to linalol in the chromatogram obtained with the reference solution.
- vi. **Determination of MIC:** The following concentration prepared using nutrient broth, 40, 35, 30, 25, 20, 15, 10, 7.5, 5, 2.5 g/dL. Tween-80 was added all assay at final concentration of 0.001% (v/v) to enhance oil solubility [9]. The MIC was defined as the lowest concentration of coriander oil preventing visible growth. All broth dilution tests were performed at least twice, if results varied tests were repeated and model selected.
- vii. **Disk diffusion method:** 0.1mL of 10<sup>8</sup> cell/mL of each microbe used in this experiments onto Nutrient agar except

*Streptococcus pyogenes* onto Brain-Heart Infusion broth (BHI) [11]. The inocula was spreaded using glass spreader or sterile cotton swab. To study the effect of coriander oil in microbes growth, we prepared filter paper disks (whattman no.1) saturated with different concentrations of coriander oil by adding 0.1 mL for each concentration to a container contains 10 sterilized disks (4mm), then the cultures inoculated at 37°C for ( 14-16)hr. The following antibiotic disks Tobramycin, Cefalexin, Gentamycin and Ampicillin were utilized as a positive control to the microbes.

### Results and Discussion:

In the following study, extraction procedure was conducted by steam distillation, the best method for extraction of the essential oil containing hydroxyl groups or other polar groups via formation oxonium extraction system, Optimum extractant was estimated depending on the quantity of linalool that extracted by the following solvents; ethanol, ethyl acetate, methanol and chloroform It was concluded that ethanol is the best extractant, table (1).

The antibiotic activity of coriander oil was tested against the growth of different microorganisms by estimation of MIC as shown in table (2). Coriander oil (linalool oil) had an inhibitory effect against the growth of *Staphylococcus aureus*, *Eschirichia coli*, *Klebsiella pneumonia*, *Streptococcus pyogenes* and *Candida albicans* at the concentration of 40%, 35%, while at the concentration of 30%, 25% the inhibitory effect was observed against *Staphylococcus aureus*, *Eschirichia coli* and *Klebsiella pneumonia*. At the concentration of 20% and 15% only *Staphylococcus aureus* growth was inhibited. No inhibition effect was observed against all

microorganisms at the concentration of 10%, 7.5%, 5%, 2.5%. The coriander oil had no inhibitory effect on the growth of *Pseudomonas aeruginosa* and *Proteus vulgaris* at all concentrations (40, 35, 30, 25, 20, 15, 10, 7.5, 5, 2.5)%, table (3).

**Table (1): Percent Extraction for Coriander oil with various extractant. (20 g of powdered fruits *Coriandrum sativum*)**

Solvent (Extractant)	Extracted coriander oil (mL)	Extraction Percent %(v/w)
<i>Ethanol</i>	14.4	72%
<i>Ethyl acetate</i>	07.4	37%
<i>Methanol</i>	13.0	65%
<i>Chloroform</i>	12.0	60%

**Table (2): MIC results for six bacterial isolates and one yeast isolate**

Microorganisms	Type of Microorganisms	Origin of specimen	Concentration of oil in gm/dl
<i>S.aureus</i>	Pathogenic	Mouth	15
<i>E.coli</i>	Pathogenic	Mouth	25
<i>K. pneumonia</i>	Pathogenic	Mouth	30
<i>P. aeruginosa</i>	Pathogenic	Wound	R
<i>P. vulgaris</i>	Pathogenic	Urine	R
<i>S. pyogene</i>	Pathogenic	Mouth	35
<i>C. albicans</i>	Pathogenic	Mouth	30

**Table (3): Antimicrobial activity of coriander oil depending on inhibition zones**

Microorganisms	40% <sup>(1)</sup>	35%	30%	25%	20%	15%	10%	7.5%	5%	2.5%
<i>S.aureus</i>	++++ <sup>(2)</sup>	+++	+++	++	+	+	R <sup>(3)</sup>	R	R	R
<i>E.coli</i>	+++	++	+	+	R	R	R	R	R	R
<i>K. pneumonia</i>	+++	++	+	+	R	R	R	R	R	R
<i>P. aeruginosa</i>	R	R	R	R	R	R	R	R	R	R
<i>P. vulgaris</i>	R	R	R	R	R	R	R	R	R	R
<i>S. pyogene</i>	++	+	R	R	R	R	R	R	R	R
<i>C. albicans</i>	+++	++	+	R	R	R	R	R	R	R

<sup>(1)</sup>: Concentration in g/dL(%); <sup>(2)</sup>: Each (+) inhibition zone size between 2-10 millimeter, (++) 11-21mm, (+++) 22-30mm and (++++) > 30 mm; <sup>(3)</sup>: (R) represents Resistant to oil activity.

The results show the inhibitory effect of coriander oil was against G<sup>+</sup> bacteria, G<sup>-</sup> bacteria and yeast, *Pseudomonas aeruginosa* and *Proteus vulgaris* were resistant to antibiotic activity of coriander oil at all concentrations may be because of their highly resistant against many antibiotics such as garamycin, carbancillin, chloramphenicol, and tobramycin. *Streptococcus pyogenes* was sensitive to the activity of coriander oil only at 40% and 35% this may be due to more than 20 enzymes and toxins produced that increased *Streptococcus pyogenes* virulence and resistance against antibiotics [11].

Ayfer and Tefi [12] studied the antimicrobial activity of the ethanolic extract of coriandrum sativum seeds and there results shows no antimicrobial effects against *Bacillus brevis* FMC3, *B.cereus* EU, *B.megaterium* D3M32, *B.subtilis* IMG22, *B.subtilis* var. *niger* ATCC 10, *Enterococcus faecalis*, *Klebsiella pneumonia* FMC 5, *Listeria monocytogenes* SCOTT A, *Micrococcus luteus* LA2971, *Mycobacterium smegmatus* RUT, *Pseudomonas aeruginosa*, *Staphylococcus aureus* ATCC25923 and *Yersinia enterocolitica* 0:P41797. but in 1998 Baratta et.al. [13] and in 2001 Elgayyar et.al. [14] and Larran et.al. [15] studied *C.sativum* and observed that the essential oil of coriander inhibited microorganisms. In 1999 Cowan [16] showed that coriander have an activity against bacteria and fungi but did not confirm the active compound.

Ethanolic extract of many Indian medicinal plants containing Phenols, tannins, flavonoids, alkaloids as major active constituents resulted with antimicrobial activity against *Staphylococcus aureus*, *Eschirichia coli*, *Bacillus subtilis* and *Candida albicans* [17]. In general depending on the site of action pharmaceutical studies of antimicrobial classified into:

1. drug that inhibit cell wall synthesis.
2. drug that inhibit nucleic acids synthesis.
3. drug that inhibit proteins synthesis.
4. drug that affecting cytoplasmic membrane. [18,19]

Frequently antimicrobial studies of plant extracts are not followed by investigations of the molecular mode of action, this regrettable because the microbial agents isolated from higher plants may acts as regulator of intermediary metabolism by activating or

blocking an enzyme reaction, removing or neutralizing an inhibitor influencing nutrient uptake from the medium, acting as depressor of or otherwise affecting enzyme synthesis of nuclear or ribosomal level, changing membrane structure, or substituting a limiting factor in intermediary metabolism [20].

Testing and evaluation of antimicrobial activity of coriander oil was difficult because of their water insolubility, complexity and instable activity; and thus explain high concentrations of MIC.

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