

Phytochemical Screening and Antibacterial Activity of Oil Extracted from the Seeds of the *Corandia Savitam* Plant

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Abstract

The *Corandia Savitam* plant has been known since ancient times because of its preventive and curative benefits against diseases, as well as its various uses in flavouring foods. And likewise, because includes beneficial and desirable effects that provide bioactive antimicrobial potential. In this study, the qualitatively phytochemical screening results of the Aqueous-Ethanollic and n-hexane oily extracts indicated that tannins, phenols, saponins, cardiac glycoside, carbohydrates and terpenoids were present in very high concentrations, while phlobatannins, alkaloids, flavonoids, proteins, quinines and oxalate were present in a moderate concentration in its oily extracts. While the quantitative results of the oil extractive revealed that the value yields for each of the oil aqueous-ethanollic and n-Hexane extracts of *Coriandrum Sativum* seed were 56.4 and 70.6%. Consequently, the best quantity of extractive value oil crop was the oil extracted by an n-Hexane solvent which was higher than the extractive value crop of oil extracted by water-alcohol as a solvent. Whereas, for each of tannins, alkaloids, flavonoids and saponins were 43.0, 57.2, 63.4 and 74.3% respectively. However, the results of the biological activity of the bout oil extracts of the *Coriander Sativum* plant seed were as follows: The Aqueous-Ethanollic oil extract against each of *Escherichia Coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella* 15, 13, 14 and 17 mm, correspondingly, even though the n-Hexane oil extract was 16, 15, 12 and 13 mm, respectively. Thus, in compression of these results. Furthermore, when comparing these results with the effects of antibiotics.

Keywords: *Coriandrum Sativum* Seeds; Phytochemical Analysis; Aqueous-Ethanollic Oil Extract; n-Hexane Oil Extract; Antibacterial Activities

Introduction

Coriander Sativum originates in Africa, Europe and Asia as well as is cultured in the Mediterranean homelands it is an anniversary herb and to the *apiaceous* family it belongs, it is also known locally as "kammon" in Libya. This herb is considered an amazing herb that behaves as a medicinal and is a commonly utilised as

a spice in cooked foods [1]. *Coriander Sativum* owns numerous phytochemicals or especially secondary metabolites which are of substantial significance in the protection mechanisms of the herb itself or for humans such as antipyretic, tonic, laxative, diuretic and carminative properties. Botanical medicine has achieved health benefits in various systems of traditional medicine from ancient

times until these days which describes nature including its plants as the most comprehensive and oldest pharmacy ever phytochemical components like saponins, flavonoids, tannins, alkaloids, phenols and many more aromatic combinations are considered secondary metabolites of plants that conform to defense against several insects and microorganisms, coumarin compounds are likewise known to function against gram-positive bacteria and it is formed in carrots in reaction to a fungal disease which may be attributed to their antimicrobial activity [2], the antimicrobial effects of saponins compounds are expected to their capability to induce leakage of proteins and certain enzymes from the cell [3]. While steroids have been noted to have antibacterial properties, the association between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically assistant with membrane lipid and exerts their action by causing leakages from liposomes [4].

Objectives of this study

The objective of this study is the preliminary qualitative detection of the biochemical ingredients of *Coriandrum Sativum* seeds as well as quantification of some of these phytochemicals constituents and then testing its bioactivities against four types of pathogenic bacteria.

Materials and Methods

Period and place of the research

This research study was implemented from February to April 2021 in the Organic Chemistry Research Laboratory of the Science College Alkhums, El-Mergib University Alkhums Libya.

Preparation of *Coriandrum sativum* seeds for oil extraction

Fresh *Coriandrum Sativum* seeds were obtained from local farms Surrounding Alkhums city, Libya. The *Coriandrum Sativum* seeds were washed with tap water and then with distilled water, to remove dust and anything unwanted. Then, to remove the excess water from it, it was left on plastic sieves at room temperature and 24 hrs. In addition, for more drying shade-dried for several days and in between observed for microorganisms or fungal infections.

Extraction of oil from the dried *Coriandrum Sativum* seeds

Far ahead, the dried *Coriandrum Sativum* seeds were ground to a coarse powder and each 30 g of the same underwent Soxhlet Extractor with 300 ml of suitable solvents (for each of n-Hexane

and 1:1 Aqueous-Ethanol solution 50%), for 5 hrs. The yield of oil was calculated after the elimination of the solvents by Rotary Evaporation at 40°C with 80 RPM and the oily extract was stored in a refrigerator at 4°C for further investigations.

Determination of *Coriandrum sativum* seed oil extractive value

The percentage yield of all oily extracts of the *Coriandrum Sativum* seed was extractive on a dry weight basis and was calculated as follows:

$$\text{Oily Yield (\%)} = \left[\frac{\text{EW}}{\text{DW}} \right] \times 100$$

Where EW is the weight of the extract after solvent evaporation and DW is the dry weight of a coarse powder of *Coriandrum Sativum* seed used for extraction.

Phytochemicals (Qualitative and quantitative screening)

A phytochemical investigation was carried out in this research, where, the oil of Aqueous-Ethanol and n-Hexane extracts, were used for the phytochemical screening of *Coriandrum Sativum* seeds oil and diverse tests existed achieved on this extract.

Phytochemical screening (Qualitative) [5]

Tannins test (Alcoholic ferric chloride solution, FeCl₃)

1 ml of the test sample was mixed well with 10% alcoholic ferric solution at room temperature. The appearance of the dark blue or greenish colour of solution is evidence of the presence of tannins.

Phenols test (Aqueous ferrous chloride solution, FeCl₂)

5 ml of the test sample was mixed well with the aqueous ferrous chloride solution of 5%. The appearance of the dark black or blue colour solution is evidence of the presence of phenols.

Phlobatannins test (Precipitation)

5 ml of the test sample was mixed well with 5 ml of aqueous Hydrochloric Acid (HCl) 1%. Then, heated. The appearance of the deposition of a small red precipitate which is the laying down of sediment is indicating the presence of phlobatannins.

Alkaloids test (Wagner's)

5 ml of the test sample was mixed well with 4 to 5 drops of Wagner's reagent. The forming of reddish-brown precipitates is indicating the existence of alkaloids.

Flavonoids test (Alkali, Na OH)

5 ml of the test sample was mixed well with 5-6 drops of the Sodium Hydroxide solution (Na OH) 20%. The appearance of the strong yellow colour during the mixing became colourless when diluted HCl was added to it. It is indicating the existence of flavonoids.

Saponins test (Foam)

5 ml of the test sample was mixed well with 10 ml of water and then shaken vigorously. The formation of persistent foam in the tube indicated the presence of saponins.

Cardiac glycosides test (Killer killiani)

5 ml of the test sample was mixed well with 10 ml of Ethanoic Acid or Glacial Acetic acid (CH_3COOH). Then 2-3 drops of ferric chloride solution were added, also mixed and followed by added 5 ml of concentrated Sulphuric Acid (H_2SO_4). The formation of a greenish or brown ring at the interface indicated the presence of Cardiac Glycosides.

Carbohydrates test (Molisch's)

5 ml of the test sample was mixed well with 2-3 drops of Molisch's reagent. Then, added 5 ml of concentrated sulfuric acid. Left to stand for 5 minutes. Then, 5ml of distilled water was added. The appearance of violet or red/dull at the interphase of two layers indicated the presence of carbohydrates.

Amino acids and proteins test (Ninhydrin)

5 ml of the test sample was mixed well with 5 drops of Ninhydrin solution (2,2-dihydroxyindane-1,3-dione) ($\text{C}_6\text{H}_4(\text{CO})_2\text{C}(\text{OH})_2$). Then heat in a water bath at 90 °C for 4-5 minutes. The appearance of purple colour indicated the presence of proteins in the solution.

Quinines test (Concentrated Hydrochloric Acid (HCl))

5 ml of the test sample was mixed well with 10 ml of concentrated hydrochloric acid. The appearance of the yellow colour indicated the presence of quinine.

Oxalates test (Ethanoic Acid Glacial)

5 ml of the test sample was mixed well with 4-5 drops of the ethanoic acid glacial. The formation of greenish-black colorations indicated the existence of oxalates.

Terpenoids test (Salkowski)

5 ml of the test sample was mixed well with 2ml chloroform or (Trichloromethane (CHCl_3)). Then sulfuric acid was added very carefully to the wall of the test tube. The formation of layers of reddish-brown colour indicated the presence of terpenoids.

Phytochemical screening (quantitative) [6-9]

Determination of tannin

8g of the fresh *Coriandrum Sativum* seeds was added 8ml of distilled water with well mixing and then shaken for 1hr. Then was filtered off. And after that 8ml of this filtrate was mixed with three 7ml of 0.1m FeCl_3 in 0.1N HCl 0.008M of potassium ferrocyanide in a test tube. Then within 10 minutes, the absorbance was measured in a spectrophotometer at 120nm wavelengths. While a blank sample was prepared and the colour was developed and read at the exact wavelength. A standard was prepared to use tannic acid to obtain 100ppm and estimated.

Determination of Alkaloid

5g of the fresh *Coriandrum Sativum* seeds was mixed well with 200 ml of 20% acetic acid in ethanol and covered to stand for 4 hrs. This was filtered and the extract was concentrated via a water bath to one-quarter of the unique volume. Ammonia solution or ammonium hydroxide solution [NH_4^+] [OH^-] was then added dropwise to the extract until the preparation was complete. The whole solution was allowed to settle down and the precipitate was collected by filtration and weighed.

Determination of flavonoid

5g of the fresh *Coriandrum Sativum* seeds was extracted repeatedly with 50ml of 80% aqueous methanol at room temperature. Then filtered off. After that, the filtrate was well along relocated into a crucible and evaporated to dryness over a water bath and then was weighed.

Determination of saponin

5g of the Fresh *Coriandrum Sativum* seeds was mixed with 50ml of ethanol solution and 20% (20ml Ethyl Alcohol and 80ml of Distilled Water v/v). After that, the mixture was heated over a water bath for 4 hrs., with continuous stirring at about 60°C. Then was filtered and the residue was re-extracted with an additional 50ml of ethanol solution of 20%. The combined extracts were reduced to

25ml over a water bath at 90°C. The concentration was transferred into a separatory funnel and 5ml of Diethyl Ether (Et₂O) or ether (C₂H₅)₂O was added and shaken vigorously. The aqueous layer was regained while the ether layer was cast off. The purification process was repeated; 15 ml of a primary alcohol 1-Butanol, but-1-ol or n-butanol (C₄H₉OH) was added. The combined n-butanol extracts were washed two times with 4ml of aqueous sodium chloride solution 5%. The residual solution was heated in a water bath; after evaporation, the samples were dried and weighed.

Antibacterial activity

In vitro carried out the microorganism tests of the aqueous-ethanolic and n-Hexane oil extracts of *Coriandrum Sativum* seed against each of Gram-negative Bacterium (*Escherichia Coli*, *Salmonella typhimurium*, *Klebsiella*), and Gram-positive Bacterium (*Staphylococcus aureus*).

Culture medium and inoculums

The stock cultures of microorganisms utilised in this research study were carried out on plate count agar slants at 4°C. The inoculum was prepared by suspending a loop full of bacterial cultures into 10mL of nutrient broth and was incubated at 37°C for 24 hours. After that, Mueller Hinton agar (MHA) (Merck) was sterilized in a flask and cooled to 45-50°C was spread by pipette 20mL into an individually sterile Petri Dish and stirred to disperse the medium homogeneously. About 0.1mL of bacterial suspension was taken and poured into Petri plates having 20mL nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were applied to obtain a consistent effects culture.

Antibacterial activity assay (Disk diffusion method) [10]

The agar Disk Diffusion Method was used for the antimicrobial assessments. Disks of 6mm (0.6cm) diameter saturated with 50µL of the aqueous-ethanolic and n-Hexane oil extracts of *Coriandrum Sativum* seed, separately, were placed on the inoculated nutrient agar medium, to each plate. And two disks were also saturated distinctly with 50µL of appropriate solvents (pure aqueous-ethanolic and pure n-Hexane, individually), which served as negative controls. Then plates were incubated at 37°C for 24 hrs. and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic medicine such as Gentamicin Sulphate, Ofloxacin and Ciprofloxacin were also screened under similar conditions for comparison. An extract was confidential as active when the diameter of the inhibition was equivalent to or larger than 6mm. The experiments were made in

triplicates. The results were obtained using the following formula

$$\text{Inhibition value} = \text{Inhibition diameter (mm)} - (\text{Disk diameter (6mm)}/2)$$

Results and Discussion

Physicochemical property	Aqueous- Ethanolic Extract	n-hexane extract
Color	Greenish brown	Greenish brown
Odour	Pleasant	Pleasant
Taste	Slightly spicy	Slightly Spicy
Smell	Aromatic	Aromatic

Table 1: Physicochemical properties of the aqueous-ethanolic and n-Hexane oil extracts of *Coriandrum Sativum* seed.

Table 1 showed some of the physicochemical properties of the aqueous-ethanolic and n-Hexane oils extracts from *Coriandrum Sativum* seed, where the colour of bout extracts was greenish brown, the Odour was pleasant, the Taste was slightly spicy and the smell was aromatic.

Phytochemical screening (Qualitatively)

Constituents	Test and Reagent	Extracts Type	
		Aqueous- Ethanolic	n-Hexane
Tannin	Ferric chloride	+++	+++
Phenol	FeCl ₂	+++	+++
Phlobatannins	Precipitation	++	++
Alkaloid	Wagner’s	++	++
Flavonoid	Alkali, Na OH	++	++
Saponin	Foam	+++	+++
Cardiac glycoside	Killer killiani	+++	+++
Carbohydrates	Molisch’s	+++	+++
Protein	Ninhydrin	++	++
Quinine	HCl	++	++
Oxalate	Ethanoic Acid Glacial	++	++
Terpenoids	Salkowaski	+++	+++

Table 2: Phytochemical screening of the aqueous-ethanolic and n-Hexane oil extracts of *Coriandrum Sativum* seed.

+++ = Abundance, ++ = Moderate, + = Low, - = Absent

As showed in table 2 the screening results of the Aqueous-Ethanollic and n-hexane oil extracts indicated that tannins, phenols, saponins, cardiac glycoside, carbohydrates and terpenoids were present in very high concentrations, while phlobatannins, alkaloids, flavonoids, proteins, quinines and oxalate were present in a moderate concentration in crude extracts. Accordingly, by reason of the presence of these secondary metabolites, coriander stands as a potential herb for anticancer, antibacterial, antiviral and antifungal therapy. Most medicinal plants including their seeds have phytochemicals or substantial secondary metabolites where

that act as a natural defense system for host plants also these are naturally ensuing, biologically active chemical constituents in plants [11]. These bioactive compounds have been a sign for humans from antiquity to the present time of the importance of the plants, whether it was ancient through tests as a treatment or nowadays by detection, purifying and identification by using advanced analyzers techniques, then used as dyes, flavor compounds, fragrances and as pharmaceuticals.

Phytochemical Screening (Quantitatively)

Plant's name	Extract's Type	Oil Extractive value (%)	Tannins (%)	Alkaloids (%)	Flavonoids (%)	Saponins (%)
<i>Coriandrum Sativum</i>	EtOH/H ₂ O	56.4	43.0	57.2	63.4	74.3
	n-Hexane	70.6				

Table 3: Results of the quantitatively evaluation of the aqueous-ethanollic and n-Hexane oil extracts of *Coriandrum Sativum* seed.

As showed in Table 3 the oil extractive value yields for each the Oil aqueous-ethanollic and n-Hexane extracts of *Coriandrum Sativum* seed were 56.4 and 70.6%. Consequently, the best quantity of extractive value oil crop was the oil extracted by an n-Hexane solvent which was higher than the extractive value crop of oil extracted by water-alcohol as a solvent. While for each of tannins,

alkaloids, flavonoids and saponins 43.0, 57.2, 63.4 and 74.3%. Usually, a quantitative evaluation is carried out to determine the standards for primary standards for crude medicines, in order to have preliminary information that is very essential for obtaining a high-quality drug.

Antimicrobial activity

Plant's name		Type of bacteria, zone of inhibition (mm)				Antibiotic
<i>Coriandrum Sativum</i>	Type of Extracts	<i>Escherichia Coli</i>	<i>Salmonella typhimurium</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella</i>	
	EtOH/H ₂ O	15	13	14	17	
	n-Hexane	16	15	12	13	

Table 4: Results of the antibacterial evaluation of the aqueous-ethanollic and n-Hexane oil extracts of *Coriandrum Sativum* seed.

Aqueous-Ethanollic extract = EtOH/H₂O.

As showed in t able 4 and diagram 1 results of the biological activity results of the two oil extracts of the *Coriander sativum* plant seeds that they were as follows: The Aqueous-Ethanollic oil extract against each *Escherichia Coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella* 15, 13, 14 and 17 mm, correspondingly, while the n-Hexane oil extract was 16, 15, 12 and 13 mm, respectively. So, in compression of these results. Furthermore, from table 4 and diagram 1, when comparing these results with the effects of antibiotics, these results, in general, are very good and close,

particularly the consequences of the aqueous-ethanollic and hexane extracts. This properties that it is possible to use the *Coriandrum Sativum* seeds for the prevention and treatment of many diseases. Consequently, the antibacterial examinations given in table 2 and as shown in figure 1 reveal the very good antibacterial potential of the *Coriandrum Sativum* Crude Aqueous-Ethanollic extract against *Escherichia Coli* 15, *Salmonella typhi* 13, *Staphylococcus aureus* 14 and for *Klebsiella* 17 mm, respectively. The existing chemical components that are products of metabolism in plants have an

important and effective role that the plant uses to defend itself or humans as vital and effective compounds against many microbes. For example, carbohydrate macromolecules, which consist of the elements of water and carbon, as these compounds are considered polar compounds and easily converted into glucose, which is used as a source of energy. While tannin is regarded as a food yield in plant vegetables. Furthermore, tannins lower bacterial propagation via blocking main enzymes in microbial metabolism [12,13]. Thus, this plant contains a higher level of tannins which might labor as an effective antimicrobial drug. Terpenoids are very potent compounds and their presence in abundance in extracts and this high amount of it indicates a strong ability to act as an antioxidant agent, which makes the plant widely important in drug discovery for the treatment of diseases since these compounds have strong antimicrobial activity, and which makes it a barrier to its reproduction.

In Libya, the *Coriandrum Sativum* seed which is from the family Apiaceae very known to use as a home treatment, medicine for health-beneficial effects, tonics and tranquillizers, and also, especially for complications such as hypertension (high blood pressure) and Hyperglycemia (high blood glucose).

Furthermore, the *Coriandrum sativum* seed is beneficial in the treatment of ulcers, cardiovascular diseases, common colds, and diarrhea, and as well as these seeds are often used as a food flavorings agent, and specifically used in preparing and making many types of bread and sweets. it is likewise, used as a folk treatment, notably for complaints of the digestive tract, concerning indigestion, nausea and flatulence too.

Conclusion

The essential oil obtained from *Coriandrum sativum* seed is rich in phytochemical constituents and yields many bioactive components with a variety of effectiveness, and these phytochemicals such as tannins, quinones, phenols, and terpenoids. These natural chemical components had an effective role in combating and killing the bacteria used in this research

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Conflicts of Interest

The authors declare no conflict of interest.

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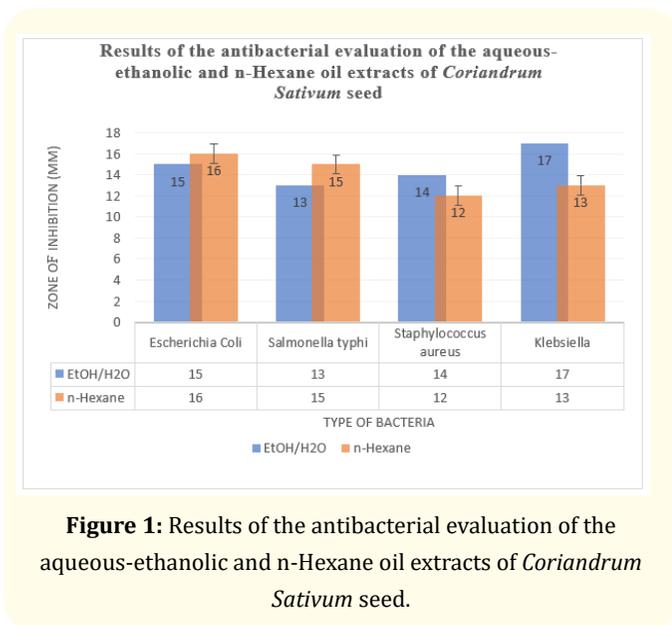


Figure 1: Results of the antibacterial evaluation of the aqueous-ethanolic and n-Hexane oil extracts of *Coriandrum Sativum* seed.

Commonly, Apiaceae family plants are utilised for medical objectives and also used for food flavoring. And because it has important medicinal properties is mainly utilised in pharmaceutical manufacturing throughout the world. Consequently, it evolved as a significant medicine for the remedy of many diseases such as cough, respiratory disorders, hay fever, vomiting, urethritis, allergies, pain, cystitis, diabetes, urinary tract, cancer, digestive and cardiovascular system [14,15].

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