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Phytochemical study and bioactivity of solvent extracts on *Coriandrum sativum*

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Abstract

The aim of the study was to know the bioactivity and phytochemical study of various methanol, acetone and benzene solvent extracts on *Coriandrum sativum* against bacteria and fungus. The extract of plant material on various solvents was tested against bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogens, Bacillus subtilis, Bacillus cereus, Bacillus megatarium, Proteus vulgaris, Salmonella typhi, Salmonella typhi* A, *Salmonella typhi* B and fungus such as *Aspergillus niger and Rhizopus* by using well diffusion method. The methanol extract of *Coriandrum sativum* was found to be more effective when compared with other solvents like acetone, benzene. The preliminary phytochemical analyses showed that the extracts contain Alkaloids, Flavanoids, Tannins, Saponin, Tarpenoids, Carbohydrates and Sterols. Therefore we can use *Coriandrum sativum* as natural antimicrobial in industrial food & drugs.

Keywords: *Coriandrum sativum*, microorganisms, phytochemical, antimicrobials.

Introduction

Coriandrum sativum (coriander) is considered as a spice and herb. It is cultivated mostly in the winter or summer as an annual crop and grown in black soil. Herbs have an important constituents use as a reduce food spoilage and control growth of food-borne pathogens. (Wong and Kitts;2005). The coriander leaves and seed contain antimicrobial and phytochemical activities and have a stronger effect against gram negative bacteria like *Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogens, Protease vulgaris, Salmonella typhi Salmonella typhi* A, *Salmonella typhi* B. and gram positive bacteria like *Bacillus subtilis, Bacillus cereus, Bacillus megatarium, Staphylococcus aureus.*(Wangensteen *et al.*, 2004).

The species of coriander have nutritional constituents like water, protein, fat, carbohydrates, calcium, phosphorus, sodium, potassium, iron, Vit –A, food energy & ash. It contains various phytochemical constituents like flavanoids, alkaloids, tannins, saponin, terpenoids, sterol, carbohydrate. Besides its various nutritional benefits, it is also well known for its health or medicinal benefits as antimicrobial agent. (Rattanachaikunsopon and Phumkhachorn;2010).

The medicinal plant has various sources of drugs. Various parts of the plant is used to extract as a raw drugs and they have varieties of medicinal properties. Some of the raw drugs are collected in smaller quantities by the local market for commersial use, many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries.

It is useful in urethritis, cystitis, urinary tract infection, urticaria, rash, burns, sore throat, vomiting, indigestion, cough, allergies, hay fever, dizziness and amoebic dysentery. (Pathak *et al.*, 2011).Fresh juice of coriander is extremely advantageous as it helps in curing many deficiencies related to vitamins and iron. Its juice is beneficial in curing many diseases. Fresh leaves can also be eaten as such because of various health benefits. (Bhat *et al.*, 2013). It may cause narcotic effect when taken in large quantities. (Singh *et al*., 2015). Medicinal plant have antimicrobial potential for therapeutic use which decreases the side effects. (Duncan *et al.*, 2012).

Materials and Methods

Materials used in the study

Various medias like, Nutrient agar medium, Nutrient broth medium, Sabouraud's Dextrose broth and Sabouraud's Dextrose Agar (SDA), were used in the study. Also various reagents like, Ferric chloride, Sodium hydroxide, Conc. HCl, Methanol, Acetone, Benzene, Ethanol, Glacial acetic acid, DMSO, Ninhydrin were used in the study.

Method

Plant material

The plant materials *Coriandrum sativum* were collected from local market of Vapi, Gujarat, India for this study.

Preparation of the Extraction (Dash *et al*., 2011)

Plant materials were washed with distilled water in order to remove any dirt particles present on the surface and were air dried at room temperature, then made to powder form. This powdered samples with concentration (10g/50ml) in methanol, acetone and benzene were kept overnight at room temperature. The extract from these solvents were soaked and evaporated under pressure.

Microorganisms

The bacterial species used for the test were *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogenes, Bacillus subtilis, Bacillus cereus ,Bacillus megatarium, Proteus vulgaris, Salmonella typhi, Salmonella typhi* A, *Salmonella typhi* B. The fungus species used for the test were *Aspergillus niger* and *Rhizopus.*

Maintainance and preservation of culture

Various non pathogenic organisms were procured from MTCC Chandigharh, India, all the cultures were maintained by sub culturing on nutrient agar slant and stored at 4˚C in refrigerator.

Culture media and Inoculum preparation

The different bacterial strains like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogens, Bacillus subtilis, Bacillus cereus,Bacillus megatarium,Protease vulgaris, Salmonella typhi, Salmonella typhi* A, *Salmonella typhi* B were innoculated in nutrient broth at 37 ^{O}C for 24 hours also various fungus species like *Aspergillus niger* and *Rhizopus* were inoculated in Sabouraud's Dextrose Agar (SDA) & broth at room temperature for 72 hours.

Phytochemical Test

The phytochemical screening was done for flavonoids, terpenoids, sterols, carbohydrates, tannins, and saponin according to study and various phytochemical tests were carried out as follows. (Lalitha and Raphael, 2012).

Test for alkaloids:

Take 0.5ml of plant extract and dilute to 10ml with acid alcohol, boil and filter, 5ml of the filtrate was added into 2ml of diluted ammonia. Then 5ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. Mayer's reagent was added & the formation of a cream or reddish brown precipitate was regarded as positive for the presence of alkaloids.

Test for flavonoids

NaOH Test

Take 2 ml of plant extract and treat with few drops of aqueous NaOH and few drops of HCl Formation of yellow orange colour indicates the presence of flavonoids.

Test for tannins

Ferric Chloride Test

Take 2 ml of plant extract and add 1 ml alcohol and treat with 1 ml of neutral ferric chloride solution.Observe for formation of blue or greenish colour solution which indicates the presence of tannins.

Test for saponins

Foam Test

Take 2 ml of plant extract, shake it vigorously. Add 3 ml water and observe for the formation of foam which is stable for 3 – 5 min. It indicates the presence of saponins.

Test for quinines

Take few drops of the plant extract and treat with 2 ml of Conc. HCl. Observe for the formation of yellow colour precipitate which indicates the presence of quinones.

Test for carbohydrates

Molisch's Test

Take few drops of Molisch's reagent. Add to 1 ml of plant extract and this was then followed by addition of 1ml of Conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5ml of distilled water. Formation of red or dull violet colour at the interphase of the two layers was a positive test which indicates the presence of carbohydrates.

Test for terpenoids

Salkowski Test

Take 0.5ml of the plant extract was added 2 ml of chloroform & Conc. H_2SO_4 (3 ml) was carefully added to form a layer. a reddish brown colouration of the interface indicates the presence of terpenoids.

Test for sterols

H_2SO_4 Test

Take 1ml of plant extract and treat with few drops of ethanol and 1 ml of H_2SO_4 and observe the formation of violet or green colour which indicates the presence of sterols.

Antibacterial activity

The plant extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic tetracycline (100mg/ml) in vitro by well diffusion method. The lawn culture of test organism on nutrient agar media were used for well diffusion methods. With the help of sterile cup borer, wells were made in the inoculated plates. The extract of *Coriandrum sativum* (500 µl) was added into the well and allowed to diffuse in the agar medium. The plates were incubated at 37˚C for overnight. The antibacterial activity of the extract was determined by measuring the diameters of zone of inhibition. For each bacterial strains, controls were maintained where, pure solvents without extracts were used. (Rathabai and Kanimozhi, 2012)

Antifungal activity

The plant extracts obtained above were also screened for their antifungal activity in comparison with standard antibiotic Fluconazole (10mg/ml) in vitro by well diffusion method. The lawn culture was prepared using the test organism on Sabouraud's Dextrose Agar (SDA) for well diffusion methods. With the help of sterile cup borer wells were made in the inoculated plates. The extract of *Coriandrum sativum* (500 µl) was added into the well and allowed to diffuse in the agar medium. The plates were incubated at room temperature for 48hrs. The activity of the extract was determined by measuring the diameter of zone on inhibition. For each fungal strains controls were maintained where pure solvents were used. (Rathabai and Kanimozhi., 2012).

Results and Discussion

Phytochemical screening of *Coriandrum sativum* with different solvents extract:

Table :1 Phytochemical screening of *Coriandrum sativum*

Compound Test and Reagent		Methanol	Acetone	Benzene
Alkaloids		+	+	+
Flavanoids	NaOH Test	+	-	-
	H_2SO_4 Test	-	+	-
Tannins	Ferric chloride Test	+	+	-
Saponins	Foam Test	+	+	+
Quinones		-	-	-
Carbohydrates	Molish's Test	+	+	+
Terpenoids	Salkowaski Test	+	-	-
Sterols	H_2SO_4 Test	+	+	+

Fig 1: (A) Test for Alkaloids (B) Test for terpenoids

(A)

(B)

The above Table-1 shows the phytochemical screening of *Coriandrum sativum* with various solvents like methanol, acetone and benzene. By the preliminary phytochemical test, the methanol extract shows the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates and terpenoids but the absence of quinines. The phytochemical test in acetone extract shows the presence of alkaloids, flavanoids, tannins, saponins, carbohydrate, sterols but absence of terpenoids, quinines whereas benzene extract shows the presence of alkaloids, saponins, carbohydrates, sterols and absence of flavanoids, tannins, quinones, terpenoids.

Kumar *et al* (2014) reported that the phytochemical screening of ethanolic extract indicates the presence of alkaloids, flavonoids, saponins, carbohydrates, terpenoids and phenolic compounds and the absence of tannins and quinones. The results of our study were in accordance to the above study.The presence of wide range of phytochemical constituents indicates that the plant could be used in many ways which may be beneficial to the population. The antibacterial activity of *Coriandrum sativum* extract was showed against various microorganisms namely, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogens, Bacillus subtilis, Bacillus cereus, Bacillus megatarium,Protease vulgaris, Salmonella typhi, Salmonella typhi* A, *Salmonella typhi* B. The different solvents used for the extaction were Methanol, Acetone, Benzene. The antibiotic tetracyclin was used as positive control.

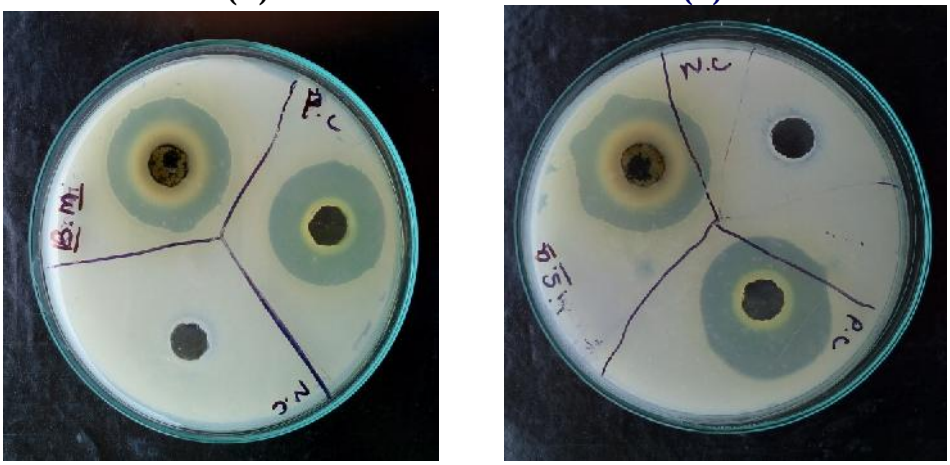
Table: 2. Antibacterial activity of *Coriandrum sativum* for methanol, acetone, and benzene extract against bacteria

Organisms (bacteria)	Zone of inhibition of Methanol ,Acetone and Benzene extract		
Eshcherichia coli	9 mm	8 mm	Nil
Pseudomonas aeruginosa	10 mm	11 mm	7 mm
Staphylococcus aureus	13 mm	12 mm	8 mm
Enterobacter aerogens	11 mm	9 mm	4 mm
Bacillus subtilis	9 mm	9 mm	10 mm
Bacillus cereus	14 mm	14 mm	8 mm
Bacillus megatarium	16 mm	10 mm	9 mm
Protease vulgaris	9 mm	Nil	Nil
Salmonella typhi	6 mm	4 mm	9 mm
Salmonella typhi A	Nil	Nil	7 mm
Salmonella typhi B	Nil	Nil	5 mm

The above Table 2 shows the zone of inhibition observed against various bacteria after period of incubation. It was found that the methanol extract was more effective against *Bacillus megatarium*, *Bacillus cereus*, *Staphylococcus aureus*, as compared to other test organisms. The zone of inhibition measured were 16 mm for *Bacillus megatarium*, 14 mm for *Bacillus cereus*, and 13 mm for *Staphylococcus aureus*. In acetone extract it was found that it was more effective against *Bacillus cereus*, *Staphylococcus aureus* and *Psedomonas aeruginosa* compared to other test oraganisms. The zone of inhibition measured were 14, mm for *Bacillus cereus*,12 mm for *Staphylococcus aureus*, 11 mm for *Pseudomonas aeruginosa*. In benzene extract it was found that it was more effective against *Salmonella typhi*, *Bacillus megatarium*, *Bacillus subtilis* as compare to other test oraganisms. . The zone of inhibition measured were 10 mm for *Bacillus subtilis*, 9 mm for *Salmonella typhi* and 9 mm for *Bacillus megatarium*. The results varied according to the study by Kumar *et al* (2014).

Fig 2: Antibacterial activity of *Coriandrum sativum* against bacteria

(A) **(B)**



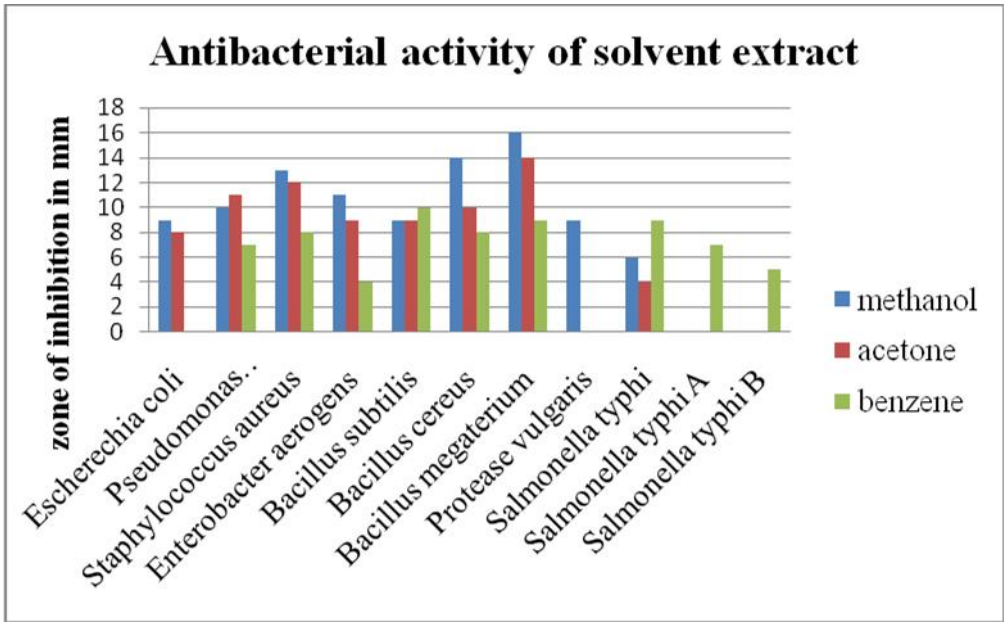


Fig.2 Antibacterial activity of methanol, acetone and benzene extracts of *Coriandrum sativum* against different organisms

V. Ratha bai *et al*. (2012) reported the efficacy of different extracts of *Coriandrum sativum*. The methanol and acetone extracts have shown better activity against these pathogenic organisms. The methanol extract was more effective against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The acetone extract was more effective against *Staphylococcus aureus* and *Kleibsiella pneumoniae*.

Table.3 Antifungal activity of *Coriandrum sativum* different solvent extract against fungus

Zone of inhibition			
Organisms (fungus)	**Methanol**	**Acetone**	**Benzene**
Aspergillus niger	10 mm	9 mm	Nil
Rhizopus stolonifer	7 mm	4 mm	Nil

The above Table-3 shows the results of antifungal activity with different solvents like methanol, acetone and benzene. The methanol extract was more effective against *Aspergillus niger*, *Rhizopus*. The acetone extract was less effective against *Aspergillus niger* , *Rhizopus*. Benzene extract did not show zone of inhibition against *Aspergillus niger*, *Rhizopus*. Standard antibiotic Fluconazole was used as a positive control. V. Ratha bai *et al*. (2012) reported that the results of antifungal activity of *Coriandrum sativum* extracts with methanol, acetone and ethanol have shown better activity against the tested organisms. The methanol extract was more effective against *Candida albicans* and *Aspergillus niger*. The ethanol extract was more effective against *Candida albicans* and *Aspergillus niger*. The acetone extract was more effective against *Candida tropicalis* and *Aspergillus niger*.

Conclusion

The preliminary phytochemical test indicates the presence of alkaloids, flavonoids, saponins, carbohydrates, terpenoids and the absence of quinones. The presence of wide range of phytochemical constituents indicates that the plant *Coriandrum sativum* could be used in a variety of ways which can be beneficial to the population. The leaves of *Coriandrum sativum* plant have strong antibacterial activity against many bacteria. From this study it can be said that, different extracts of *C. sativum* showed wide range of antibacterial activity and can be used for the medical purpose. The spectrum of antibacterial activities provides support to some traditional uses of this medicinal plant.

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