Antibacterial Activity of *Coriandrum sativum* L. Extracts (Nannan)

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Coriandrum sativum L. is consumed in human diet, is a good source of minerals and vitamins and widely distributed in Myanmar. In this study, antibacterial activities of different extracts (70% ethanol and 70% methanol) of *Coriandrum sativum* L. (whole plant) were studied on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* Ceftriaxone was used as positive control. The antibacterial activity of *Coriandrum sativum* L. extracts was determined by agar disc diffusion method. The 70% ethanolic extract showed zone of inhibition 8 mm and 70% methanolic extract showed 9 mm on *E. coli, S. aureus* and *P. aeruginosa.* Broth dilution method was used for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Coriandrum sativum* L. MIC and MBC of ethanolic extract and methanolic extracts of *Coriandrum sativum* L. were 400 mg/ml on *E. coli, S. aureus* and *P. aeruginosa.* Phytochemical analysis of *Coriandrum sativum* L. was also carried out and alkaloids, glycosides, steroids, cardiac glycosides, amino acids, starch, phenols, tannin and carbohydrates were detected, and flavonoids, saponin, protein, resins and cyanogenic glycosides were not detected. Antibacterial activity of *Coriandrum sativum* L. may be due to the presence of phenols. Therefore, this study provided useful information about antibacterial activity of different extracts of *Coriandrum sativum* L. (whole plant).

Keywords: Antibacterial activity, *Coriandrum sativum* L., *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*

INTRODUCTION

Spices and herbs have been used for many years by different cultures to enhance the flavor and aroma of foods, in preserving foods and for their medicinal value. These attributes are useful in the development of snack foods and meat products.[1] Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs and their components.[1-3] World Health Organization stated that medicinal plants would be the best source to obtain a variety of drugs.[4] *Coriandrum sativum* L. should be investigated to understand their properties, safety and efficiency.[5] Medicinal plants represent a rich source of antimicrobial agents. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. The spice, *C. sativum* is one of the plants that are known to produce essential oils with antimicrobial activity.[6] Essential oils prepared from the seeds and immature leaves of coriander inhibit the growth of *Pseudomonas fragi, Escherichia coli, Salmonella*

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DOI: https://doi.org/10.34299/mhsrj.00984

typhimurium, Listeria monocytogenes, Staphylococcus aureus and *Saccharomyces cerevisiae* in individual and mixed fractions such as essential oils of *Anethum graveolens* and *Eucalyptus dives.*[7] More studies revealed that 100 µl/mL of coriander volatile oil had antimicrobial activity on *Staphylococcus coagulase, E. coli, Streptococcus fecaelis,* and *S. aureus*.[8] The ethanolic extract of *C. sativum* L. root (50 µg/mL) showed antimicrobial effect against *S. aureus, Salmonella typhi, Bacillus cereus, Klebsiella* and *Candida.*[9] The present study is aimed to determine the antibacterial activity of different extracts of the whole plant of *C. sativum* L. on *E. coli, P. aeruginosa and S. aureus.*

MATERIALS AND METHODS

This study was a laboratory-based experimental study carried out during 2016 and 2017 at Department of Medical Research (Pyin Oo Lwin Branch), Mandalay District.

Collection of plant material

Coriandrum sativum L. was collected from Mandalay Region.

Identification of plant

Identification of plant was performed at Department of Botany, University of Mandalay by using The Flora of Ceylon.[10]

Phytochemical analysis

Phytochemical analysis of whole plant was also done by phytochemical techniques.[11]

Extraction of plant

Coriandrum sativum L. were collected and thoroughly washed with water and then air-dried for about two weeks. The whole plant was powdered and extracted by percolation method.[12] The plant sample 100 g was percolated with 1000 ml of 70% ethanol for a week in a percolator. The liquid extract containing plant constituents was filtered and evaporated on the water bath until to get constant weight and stored in desiccators. Methanolic extract of *C. sativum* L. was obtained by the same procedure.

Determination of antibacterial activity of different extracts of the whole plant of Coriandrum sativum L.

Antibacterial activity of different extracts of *Coriandrum sativum* L. was determined by agar disc diffusion technique according to modified Kirby and Bauer method.[13]

Preparation of medium

Muller-Hinton Agar was prepared according to the procedure of the manufacturers' recommendation and sterilized by moist heat at 121°C for 15 minutes. After autoclaving, 25 ml of the media was poured into 9 cm diameter petridishes and allowed to set at room temperature. It was prepared freshly before use. When the agar had solidified, the plates were dried at 50°C in the upright position in the oven with the lids tilted. The plates were then labeled.

Preparation of bacterial suspension

A few colonies of organisms from the subculture to be tested were picked with a wire loop and introduced into test tube containing peptone solution. These tubes were incubated at 37°C for 3-4 hours to produce the growth turbidity.

Preparation of impregnated disc of different plant extracts

The sterile discs, 6 mm in diameter, were spread out separately in petridishes, so that each disc was not less than 2 mm from its neighbours. They were sterilized by dry heat at 160°C for 1 hour. Different doses of ethanolic extracts of *C. sativum* L. (40 mg, 80 mg, 120 mg, 160 mg, 200 mg etc.,) were dissolved in 1 ml of 70% ethanol separately. From the different stock solutions, 20 µL of solution was impregnated to discs, respectively, and dried in the oven at 37°C to evaporate the solvent. Discs for methanol extract of *C. sativum* L. were done by the same procedure. Antibiotic disc, ceftriaxone (30 µg), was used as positive control reference standard. Disc as negative control was prepared using the same solvent employed to dissolve the plant extract.

Antibacterial susceptibility test

Antibacterial susceptibility test was determined by a standard disc diffusion technique using Muller-Hinton agar according to the recommendations of Clinical and Laboratory Standards Institute (CLSI).

A sterile cotton swab was dipped into bacterial suspension. Freshly grown liquid cultures of the test pathogens were seeded over the Muller-Hinton agar (MHA) plates with a sterile cotton swap. The swab was streaked in at least three directions through the angle of 60° over the surface of the Muller-Hinton agar to obtain uniform growth. A final sweep was made around the edge of the agar surface. After the inoculum has dried for a few minutes, the sterile filter paper discs impregnated with plant extracts were placed on the seeded MHA plates at equal distance with sterile forceps and gently pressed down to ensure contact with the medium. The plates were incubated at 37°C for 24 hours. Following overnight incubation, zones of inhibition occurred around the discs. The inhibition zones were recorded as millimeters (mm).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of antimicrobial activity that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentration (MBC) as the lowest concentration of antimicrobial activity that will prevent the growth of an organism after subculture on to antibiotic-free media. The determination of the MIC involves a semi-quantitative test procedure which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. MIC/MBC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution method and agar dilution method. Serial dilutions of the extracts were prepared from the highest concentration of 1000 mg/ml to the lowest concentration of 200 mg/ml. The test organisms are then added to the dilution of the products, incubated and scored for growth.

Determination of minimum inhibitory concentration and minimum bactericidal concentration of ethanolic and methanolic extracts of Coriandrum sativum L.

The ethanolic and methanolic extracts of *Coriandrum sativum* L. were proceeded for minimum inhibitory concentration (MIC) by broth dilution method.[14]

Different concentrations of ethanolic and methanolic extracts of *C. sativum* L. ranging from 200 mg/ml to 1000 mg/ml. were tested on different test organisms. A series of ten tubes for each test organism were prepared. Each tube contains 20 μL of test organisms in 1 ml of Muller-Hinton broth. The different dilutions of 1 ml of ethanolic extract of *C. sativum* L. were added to the tubes. The eighth tube was used as control tube which contained Muller-Hinton broth, 70% ethanol with test organisms. The ninth tube was used as test extract control and the tenth tube, test organisms only. Then, the different dilutions of methanolic extract were also done and incubated at 37°C for 24 hours. After incubation, minimum inhibitory concentration (MIC) was recorded as tube with lowest concentration at which no visible turbidity was observed. For determination of minimum bactericidal concentration (MBC), one loopful from each tube of above dilutions was streaked on Muller-Hinton agar plate and incubated at 37°C for 24 hours.

RESULTS

Plant identification

The plant was identified as *Coriandrum sativum* L. belonging to the family Umbelliferae.

Yield percents of plant extracts

The different yield percents of plant extracts of *C. sativum* L. are shown in Table 1.

Table 1. Determination of yield (%) of different extracts of *C. sativum* L.

Solvent	Weight (g)	Yield % (w/w)
70% Ethanol	100	24.19
70% Methanol	100	24.63

Phytochemical analysis

The result of phytochemical screening of *C. sativum* L. is shown in Table 2.

Table 2. Result of phytochemical test on *C. sativum* L.

Type of compound	Results	
	Methanolic extract	Ethanolic extract
Alkaloid	(+)	(+)
Carbohydrates	(+)	(+)
Flavonoids	(-)	(-)
Glycosides	(+)	(+)
Phenols	(+)	(-)
Cyanogenic glcosides	(-)	(-)
Protein	(-)	(-)
Saponin	(-)	(-)
Resin	(-)	(-)
Starch	(+)	(+)
Steroids	(+)	(-)
Tannins	(+)	(+)
Cardiac Glycosides	(+)	(-)
Amino Acids	(-)	(+)

(+) detected, (-) not detected

Antibacterial activity of different extracts of Coriandrum sativum L.

Antibacterial activity of *C. sativum* L. extracts was determined by agar disc diffusion method (Kirby and Bauer method). Both 70% ethanolic extract and methanolic extract showed zones of inhibition on *E. coli, S. aureus* and *P. aeruginosa* (Table 3).

Table 3. Antibacterial activities of different extracts of *C. sativum* L.

Test organism	Diameter of inhibition zone (mm)		
	Ceftriaxone (30 µg)	70% Etha-nolic extract	70% Metha nolic extract
Escherichia coli	20 mm	8 mm	9 mm
Staphylococcus aureus	20 mm	8 mm	9 mm
Pseudomonas aeruginosa	20 mm	8 mm	9 mm

Paper disc - 6 mm

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of C. sativum L. extracts

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *C. sativum* L. extracts were determined by broth dilution method. MIC of ethanolic extract and methanolic extracts of *C. sativum* were 200 mg/ml on *E. coli*, *S. aureus* and *P. aeruginosa*, respectively. MBC of ethanolic extract and methanolic extracts of *C. sativum* L. were 400 mg/ml on three bacteria, respectively (Table 4).

Table 4. MIC and MBC of different extracts of *C. sativum* L.

Test organism	70% Ethanolic extract		70% Methanolic extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Escherichia coli	200	400	200	400
Staphylococcus aureus	200	400	200	400
Pseudomonas aeruginosa	200	400	200	400

MIC = Minimum inhibitory concentration
MBC = Minimum bactericidal concentration

DISCUSSION

For phytochemical investigations in this study, alkaloids, glycosides, steroids, cardiac glycosides, amino acids, starch, phenols, tannin and carbohydrates were detected, and flavonoids, saponin, protein, resins and cyanogenic substances were not detected. As the phenolic compounds were most active antibacterial components against gram-positive and gram-negative bacteria[15], antibacterial properties of *C. sativum* L., may be due to the presence of phenols.

Regarding antibacterial activity of *C. sativum* L. extracts, 70% ethanolic extract showed zone of inhibition 8 mm and 70% methanolic extract showed 9 mm on *E. coli*, *S. aureus* and *P. aeruginosa*, respectively. The previous study showed that ethanolic extract showed zone of inhibition 11.17, 11.4 and 9.97 mm and methanolic extract showed 9.97, 12.17 and 9.9 mm on *E. coli* [ATCC-27853], *S. aureus* [ATCC-25923] and *P. aeruginosa* [ATCC-25923], respectively.[16] This difference may be due to the disparity of extraction methods, species of strains and resistance of organisms. According to the results, the ethanolic extract and methanolic extracts of *C. sativum* L.

have shown the antibacterial activity against *E. coli, S. aureus* and *P. aeruginosa*, respectively.

Conclusion

From phytochemical investigations, the whole plant of *C. sativum* L. had many active phytochemical compounds including alkaloids, glycosides, steroids, cardiac glycosides, phenols and tannin. However, flavonoids, saponin, protein, resins and cyanogenic glycosides did not contain in *C. sativum* L. As regard the antimicrobial activity of *C. sativum* L. extracts, 70% ethanolic extract showed zone of inhibition 8 mm and 70% methanolic extract showed 9 mm against *E. coli, S. aureus* and *P. aeruginosa.* MIC and MBC of ethanolic extract and methanolic extract of *C. sativum* L. showed the antibacterial activity against *E. coli, S. aureus* and *P. aeruginosa.* This study provides the useful information about the antibacterial activity of *C. sativum* L. and will be platform for the development of newer antibiotic drugs.

Competing interests

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

We would like to thank all staff from Bacteriology Research Division, Department of Medical Research (Pyin Oo Lwin Branch) for their technical support in conducting this research.

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