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Antimicrobial Effect of Seed Ethanolic Extract of Coriander

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The aim of the study was to screen the antibacterial activities of ethanolic and aqueous-ethanolic extracts of Coriander (*Coriandrum sativum*) on some microorganisms including *Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris*, *Escherechia coli, Pseudomonas aeruginosa, Klebsiella peunomonia, Listeria monocytogenes,* and *Candida albicans*. According to findings, both ethanolic and Aqueous-ethanolic extract of coriander seed displayed a variable degree of antimicrobial activity on different microrganisms. The ethanolic extract showed clear difference and more potent against tested microorganisms in comparison with the aqueous-ethanolic extract. It is determined that Ethanol extract revealed an elevated antimicrobial activity against *Proteus vulgaris* and *Candida albicans* whereas aqueous-ethanolic extract of spices exhibited highest activity against *Bacillus subtilis and Listeria monocytogenes*. The results obtained in the present study suggest that the ethanol extract of Coriander revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulation.

Key words: Antimicrobial effect, Extract, coriander.

Recently there has been a renewed interest in improving health and fitness through the use of more natural products. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value[1] which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices.

Coriander (*Coriandrum sativum* L.) is a well-known herb widely used as a pharmacy and food industries[2]. This plant is widely distributed and mainly cultivated for its seeds which are used for different purposes such as food, drugs, cosmetics, perfumery and medicinal uses. It is native to the Mediterranean and Middle Eastern regions and has been known in Asian countries for thousands of years[3]. The seeds of coriander

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were found in the ancient Egyptian tomb of Ramses the Second. The Egyptians called this herb the ''spice of happiness", probably because it was considered to be an aphrodisiac[4]. It was used for cooking and for children's digestive upset and diarrhea.

The coriander seeds contain 0.5-1% essential oil and are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol, and linalool. Coriander's flavonoids include quercitin, kaempferol, rhamnetin, and epigenin. Coriander also contains active phenolic acid compounds including caffeic and chlorogenic acid[5]. The seeds are mainly responsible for the medical use of coriander and have been used as a drug for indigestion, against worms, rheumatism and pain in the joints[6]. It has traditionally been referred to as an anti-diabetic[7], anti-inflammatory and recently been studied for its cholesterol-lowering effects[8]. In addition, it is also used as carminative, diuretic, tonic, stimulant, stomachic, refrigerent, aphrodisiac and analgesic.

This study was carried out to determine whether seed extract of coriander (*Coriandrum sativum*) has inhibitory activity on some pathogens and saprophytic microorganisms

MATERIALS AND METHODS

Preparation of extracts

The Fresh coriander seeds, was purchased from a local market at Riyadh, Saudi Arabia. About 100 g of fenugreek seeds were crushed in a mortar. Exactly 10 g of fenugreek seeds powder were soaked in 100 ml of 50% ethanol water with agitation at 40°C. The EtOH:H_20 extract was then filtered, evaporated under steam of nitrogen using sample concentrator model Techne DB.3 (Techne, UK). The yield of the aqueous-ethanol extract was 1.8 g. Aliquot of the extract was resolved in ethanol to a final concentration of 1.0 mg/mL.

Microorganisms

Antibacterial activity of coriander extracted was tested against 7 bacterial strains of which include Gram negative, Gram-positive reference strains and *Candida* spp. They were obtained from the Microbiology laboratory, Department of life science, faculty of science and Department of pharmaceutics and microbiology, faculty of pharmacy, King Saud University. They were; *Bacillus subtilis* ATCC 10400, *Staphylococcus aureus* ATCC 2491, *Proteus vulgaris* FMC1, *Escherechia coli* ATCC 25912, *Pseudomonas aeruginosa* ATCC 10662, *Klebsiella pneumoniae* ATCC 27736, and *Listeria monocytogenes* SCOOT A. *Candida albicans* was used for fungal activity test. All bacterial strains were stored in Brain Heart Infusion (BHI) broth with 20% (v/v) glycerol at -70°C. They were maintained as pure cultures in respective specific agar slants at 40°C.

Culture medium and inoculum:

Prior to susceptibility testing, each strain was resuspended to ensure optimal growth and purity. The tested original strains are activated formerly with the corresponding medium slant. Cell suspensions were prepared by inoculation of each bacterium into 10 ml of sterile saline solution in a test tube by inoculation loop and then vortexed and oscillated into the bacterial suspension standby. Incubation was performed at 37 °C for 24 h. Muller Hinton Agar (MHA) (Difco, USA) was prepared and cooled to 45 °C. Bacterial suspension was added into MHA to give a final concentration of 107 CFU/ml and plated out. Sabouraud's dextrose broth was used for *Candida albicans*.

Antibacterial susceptibility studies

The antimicrobial susceptibility test of the extracts were carried out by determining the zone of inhibition using disc diffusion method as described by Brooks *et al*., 2002 [9]. The strains were grown to logarithmic phase in Muller-Hinton broth and the inoculum was prepared by adjusting the turbidity of bacterial suspension to 0.5 McFarland's tube with Muller-Hinton broth. Filter paper discs of 5.0 mm diameter were prepared and sterilized by autoclaving. The quantity of 1.0g (1000 mg) of the dried extracts were dissolved in 5% dimethyl sulphoxide (DMSO) to the concentration at 200mg/ml and finally sterilized by filtration. The sterile discs (5 mm in diameter) were impregnated with 20 µl of the above mentioned extracts to achieve desired concentration of 4mg/ml. The cultures were enriched in sterile nutrients broth for 18 h at 37°C using sterile cotton swabs; the cultures were aseptically swabbed on the surface of sterile Muller-Hinton plates using an ethanol dipped and flamed forceps. The antibiotic discs

were aseptically placed on the upper layer of the seeded MHA plates for bacteria and Sabouraud's dextrose agar for Candida sufficiently separated from each other to keep away from overlapping of inhibition zones. The plates were incubated in an upright position at 37°C for 24 h. The diameters of the zones of inhibition appearing around the discs were measured to the nearest millimeter (mm) and the results were recorded. Discs less than 7 mm diameter are considered as having no antibacterial activity. Diameter between 7 and 12 were considered as moderate active and those with > 12 mm were considered as highly active. Streptomycin (10 µg/disc) and fluconazole disc (10µg) were used as positive control for bacteria and Candida respectively. 5% DMSO impregnated discs was used as negative control. All of the susceptability tests were performed in triplicate and expressed as average value.

Minimum Inhibitory Concentration (MIC)

The microdilution method was used according to the Clinical and Laboratory Standards Institute (CLSI, 2007) recommendations[10]. Plant extracts solution were separately added into wells in a final concentration 3mg/ml after dissolved in DMSO. Standardized suspensions of the test organisms (equivalent to the 0.5 McFarland) were prepared from overnight cultures in MH broth. The test organisms were inoculated using multipoint inoculators (104 CFU/spot). Control wells were also included in these experiments, and contain either MH broth only or plant extracts and MH broth without bacteria. Each plant extract was run in duplicate. The plates were incubated at 37°C for 24 h. MIC was defined as the lowest extract concentration, showing no visible growth after incubation time. 5 µl of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for tested bacteria was determined as the lowest concentration of the ginger extract inhibiting the visual growth of the test cultures on the agar plate.

RESULTS AND DISCUSSION

The beneficial health effects of extracts from many types of plants that are used as seasoning agents in foods and beverages have been claimed for centuries. In this study, the purpose was to examine the inhibitory effects of coriander seed extract, some pathogens causing food poisoning and different illnesses in humans, and some microorganisms causing spoilage in foods were used as test strains. For this purpose, the ethanolic and aqueous-ethanolic extracts of coriander seeds were tested for different microorganisms including *Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Escherechia coli, Pseudomonas aeruginosa, Klebsiella peunomonia, Listeria monocytogenes* and *Candida albicans* with disc diffusion method as in vitro. Inhibition zone diameter (DIZ) and Minimum Inhibitory Concentration (MIC) were determined against all the tested bacteria as shown in Table 1 and Table for ehanolic and aqueous-ethanolic extract respectively. The MIC results of different extracts were gotten from the extrapolation diameter zone of inhibition of the concentration. Negative control (disc containing only methanol or aqueous-ethanol) showed no zone against any bacteria. All the positive controls showed antibacterial activity against tested bacteria.

Table 1. Activity of crude ethanol extract of coriander seeds

Bacteria	DIZ Mm	MIC Mm	Pos. control mg/mL	Neg. control
Bacillus subtilis	14	32	22	-
Staphylococcus aureus	14	32	20	-
Proteus vulgaris	16	16	23	-
Escherechia coli	14	32	20	-
Pseudomonas aeruginosa	12	64	22	-
Klebsiella peunomonia	14	16	22	-
Listeria monocytogenes	14	32	20	-
Candida albicans	16	nt	Nt	-

Proteus vulgaris and *Candida albicans*

Table 1. Activity of crude aqueous-ethanolic extract of coriander seeds

Bacteria	DIZ Mm	MIC Mm	Pos. control mg/mL	Neg. control
Bacillus subtilis	10	64	22	-
Staphylococcus aureus	9	64	20	-
Proteus vulgaris	8	32	23	-
Escherechia coli	8	32	20	-
Pseudomonas aeruginosa	6	125	22	-
Klebsiella peunomonia	7	32	22	-
Listeria monocytogenes	10	64	20	-
Candida albicans	8	**nt**	nt	-

Ethanol extract of coriander seed were found sensitive to both *Proteus vulgaris* and *Candida albicans* as shown in Table 1. Crude ethanolic extracts showed varying diameter zone of inhibition with the different test organisms. It exhibited the widest inhibition zone of 16 mm. with Proteus vulgaris and *Candida albicans* followed by 14 mm. with *Escherechia coli, Klebsiella pneumonia, Listeria monocytogenes, Bacillus subtilius* and *Staphylococcus aureus*. Ethanolic extract gave lowest inhibition zone of 12mm against *Pseudomonas aeruginosa*. The MIC against the test isolates is ranged from 16-64 $mgml^{-1}$. The MIC values of ethanol extract were found to be 64 $mgml^{-1}$ against *Pseudomonas aeruginosa*, The MIC values were found 32 $mgml^{-1}$ against *Bacillus subtilis, Staphylococcus aureus, Escherechia coli* and *Listeria monocytogenes*. The MIC values were found 16 $mgml^{-1}$against *Proteus vulgaris* and *Klebsiella peunomonia* (Table 1).

Aqueous-ethanolic extract of coriander seeds was found sensitive to *Pseudomonas aeruginosa* and *Klebsiella peunomonia*. Aqueous-ethanolic extract of Coriander produced zone of inhibition of 6 and 7mm against *Pseudomonas aeruginosa* and *Klebsiella peunomonia* respectively. Aqueous-ethanolic extract produced zone of inhibition 8 mm against *Proteus vulgaris* and *Escherechia coli* and 9 mm against *Staphylococcus aureus.* However, it exhibited highest zone of inhibition (10 mm) against both *Bacillus subtilis* and *Listeria monocytogenes* (Table 2). The MIC values of aqueous-ethanolic extract were found to be in the range 32-125 $mgml^{-1}$. It was found 125 μgml^{-1} against *Pseudomonas aeruginosa*. For *Bacillus subtilis, Staphylococcus aureus* the and *Listeria monocytogenes* , the MIC values were found 64 $mgml^{-1}$. The lower value of MIC (32 $mgml^{-1}$) were measured for *Proteus vulgaris, Escherechia coli* and *Klebsiella peunomonia.*

In this study, both ethanolic and Aqueous-ethanolic extract of coriander seed displayed a variable degree of antimicrobial activity on different microrganisms. The ethanolic extract showed clear difference and more potent against tested microorganisms in comparison with the aqueous-ethanolic extract. *Proteus vulgaris* was found to be more sensitive strain then the others. On the other hand *Pseudomonas aeruginosa* was found to be most resistant bacteria against the cardamom seed. Some investigators noted that sensitivity of microorganisms to chemotherapeutics differs according to type of strain[11]. Similar results have been observed in our study[12]. Antimicrobial characteristics of the herbs are due to various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissue[13,14]. The inhibitory effect of cardamom seeds detected in the present study may be due to the presence of volatile oils.

CONCLUSION

In conclusion, our results indicated that extract of the coriander seed which was prepared using ethanol, has a strong inhibitory activity on some pathogens than Aqueous-ethanolic. According to us, using coriander as antimicrobial additives in food may be useful. The present study has revealed the importance of natural products to control resistant bacteria which are becoming a

threat to human health. This scientific study can serve as an important platform for the development of inexpensive, safe and effective natural medicines.

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