



Potentials of *Cuminum cyminum* in Microbial Control

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Abstract

Cumin is one of the important spices known for their distinctive aroma, cumin seeds are sprinkled over popular North African, Middle East, Western, Chinese, Indian, Cuban and Northern Mexican cuisines. The spice is native to middle-east, Asian region, and now grown all over the world for its flavorful seeds. The plant is the small flowering herb belonging to the family of *Apiaceae* of the genus *Cuminum*, and scientifically known as *Cuminum cyminum*. It is an important condiment and spice crop grown in India for its aromatic-seed. India is the leading country in the world in production, consumption and export of cumin. It is grown in Madhya Pradesh, Rajasthan, Gujarat, West Bengal, Assam, Kerala, Uttar Pradesh and Punjab. Cumin is used as condiment for flavor and in medicines. Though cumin is one of the important spice crops with high economic returns, it has not yet gained commercial significance in most of the areas favorable for its cultivation. RS-I variety of cumin plant is recommended for Haryana, Punjab and Rajasthan. Cumin plays an important role in human health owing to their anti-inflammatory, anti-allergic, antimicrobial, anticarcinogenic, antiviral activities. Furthermore, it is anti-diuretic and anti-oxidant.

Considering the above facts our study was carried out to observe & evaluate the effect of extracts from cumin against microbial population considering both bacterial & fungal forms. Cumin-acetone fractions were extracted with Soxhlet and considered for tests against Gram negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus*) and the fungi (*Aspergillus niger*, *Penicillium notatum*, *Mucor indicus*). All the observations showed positive results by appearance of clear zone of inhibition on cultured petri plates for bacteria. However, Work on fungal species are on progress. Further, studies are going on MIC and analysis of the extract, which may provide us new ideas in the study of bio-active agents.

Keywords: *Cuminum cyminum*, acetone, bacteria, bio-active agents.

Introduction

Cumin (*Cuminum cyminum*) is a flowering plant, belonging to the family *Apiaceae*, is a herbaceous annual plant, with a slender branched stem 20-30 cm tall. The leaves are 5-10 cm long, pinnate or bipinnate, thread-like leaflets. The flowers are small, white or pink, and borne in umbels. The fruit is a lateral fusiform or ovoid achene 4-5 mm long, containing a single seed. In India cumin is

known as 'jeera' or 'jira'. Cumin originates from Egypt, Turkistan and East Mediterranean, but it is cultivated in Iran, China, India, Morocco, south Russia, Japan, Indonesia, Algeria and Turkey (Tuncturk, R., & Tuncturk, M., 2006). In Iranian folk medicine, cumin seeds have been used to treat diarrhea, toothache and epilepsy (Zargari, A., 1989). In recent years, there are some reports regarding the anti-diabetic

activities (Talpur, *et al.*, 2005) of this herb and its effects on reductions of cholesterol, phospholipids, free fatty acids, and triglycerides in plasma and tissue (Dhandapani, *et al.*, 2002; Malini & Vanithakumari, 1987). Chemical composition of cumin seed essential oil has been extensively studied (Behera *et al.*, 2004; Hashemi *et al.*, 2008) and cuminaldehyde has been introduced as the characteristic constituent of the seed (Sowbhagya, H. B. *et al.*, 2008). Potentials of cuminaldehyde as a major agent for antidiuretic therapeutics were also reported (Lee H-S, 2005). Different studies have been conducted on the yield of cumin essential oil (Raghavan, 2007), the effect of size reduction and expansion on yield and quality of cumin seed oil was evaluated. For small batch size operations (200g), oil yield was found to be the same (3.4%) for both ground and flaked. Cumin also contains 10% fixed oil (Nostro, *et al.*, 2005). *C. cyminum* seeds are also used for treatment of dyspepsia and jaundice (Allahghadri, *et al.*, 2010), whereas, essential oil of *C. cyminum* seed exhibits diuretic, carminative, emmenagogic, antibacterial, antioxidant, cytotoxic, and antispasmodic properties (Li & Jiang, 2004). Cumin seed contains fixed oil (approximately 10%), protein, cellulose, sugar, mineral elements and volatile oil (Peter, 2001). They also contain volatile oil (1–5%) that accounts for the typical aroma of cumin seeds. The essential oil is responsible for the characteristic cumin odor. This odor and flavor are due principally to the aldehydes present. Studies of the chemical composition of cumin oil from different countries showed the presence of the following components: α -pinene (0.5%), Myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), p-menth-3-en-7-ol (0.7%), pmentha-1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β -bisabolene (0.9%), β -pinene (13.0%), P-cymene (8.5%), β -phellandrene (0.3%), D-terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol (2.8%), β -farnesene (1.1%) together with much smaller quantities of α -phellandrene, α -terpinene, cis and trans sabinene, Myrtenol, α -terpineol and phellandral (Nanasombat, & Lohasupthawee, 2005). A comprehensive and extensive study was done by Liu, Q. *et al.*

(2017) on several spices, such as clove, oregano, thyme, cinnamon, and cumin. Study results showed significant antimicrobial activities against food spoilage bacteria like *B. subtilis* and *P. fluorescens*; pathogens like *S. aureus*, *V. parahaemolyticus*, and *S. typhimurium*; harmful fungi like *A. flavus* and *A. niger*, and even antibiotic resistant microorganisms such as MRSA. Therefore, these spices could be used to decrease the possibility of food poisoning and spoilage, to increase the food safety and shelf-life of products, and to treat some infectious diseases. In another study cumin was discussed as the major antimicrobial component inhibiting growth of *B. cereus*, *B. subtilis*, *Ls. monocytogenes*, *C. freundii*, *K. pneumoniae*, *Ps. fluorescens*, *S. enteritidis*, *St. aureus*, *A. niger*, *S. cerevisiae*, *C. albicans* (Gottardi, *et al.*, 2016). India represents the world leader in the jeera production. India's production sums up to 1 to 2 lakh tons of jeera per year that makes it the leading producer in the world. The country also has the largest area allotted towards jeera production i.e. around 5.25 lakh hectares. The level of production and the total area under jeera cultivation has increased significantly during the last few years. Rajasthan is the largest cumin producing state and Gujrat is second largest state in India.

Considering the above facts our study was carried out to observe & evaluate the effect of extracts from cumin against microbial population considering both bacterial & fungal forms. Cumin-acetone fractions were extracted with Soxhlet and considered for tests against Gram negative bacteria (*Escherichia coli* MIC 2642), Gram-positive bacteria (*Staphylococcus aureus* MIC 7443) and the fungi (*Aspergillus niger* NCIM 1054, *Penicillium notatum* NCIM 745, *Mucor indicus* MTCC 3318 and *Trichoderma viride* NCIM 1053). All observation showed positive results by appearance of clear zone of inhibition on cultured petri plates for bacteria. Moreover, Works on fungal species are on progress. Further, studies are going on MIC and analysis of the extract, which may provide us new ideas in the study of bio-active agents.

Materials and Methods

Plant materials

The plants used in this study was *Cuminum cyminum* (common name: Cumin); the seed was collected from the local spice shops. After collection, 10g of each of the plant parts were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers.

Preparation of extracts

Soxhlet extraction:

The dried and powdered plant materials (10g) were extracted successively with 140ml and 190ml of the two concentration of solvents by using soxhlet extractor for 8h at a temperature not exceeding the boiling point of the Solvent (Lin, *et al.*, 1999). The concentration of solvents used for the study was 50% acetone and 100% acetone. The extracts were filtered and then concentrated to dryness. Each extract were transferred to glass vials and kept at 4° C before use.

In vitro testing of extracts for antimicrobial activity:

Antibacterial assay:

Overnight cultures of the Gram positive strains *Staphylococcus aureus* and the Gram negative strains *Escherichia coli* were prepared on nutrient agar plates. All bacterial isolates were suspended in saline to turbidity. Sterile molten nutrient agar at around 40°C was taken and seeded with different bacterial

Results

Soxhlet extraction:

Yield of the extract obtained was calculated as follows:

Table 1. Yield of the extract

SAMPLE	SOLVENT	YIELD
Cumin	Acetone (50%)	7.28%
	Acetone (100%)	10.5%

cultures and plates were prepared. After solidification 4 mm wells were prepared. In these wells solvent extracts of the peel were added. The plate was incubated overnight at 37°C. After incubation the zones of inhibition were measured and recorded. Respective solvent controls were also run simultaneously.

Antifungal Assay:

Cultures of fungal strains *Penicillium notatum*, *Trichoderma viridae*, *Aspergillus niger* and *Mucor indicus* were prepared on Potato Dextrose agar plates. All fungal isolates were suspended to turbidity. Sterile molten agar plates was taken and seeded with different fungal cultures and plates were prepared. After solidification 4 mm wells were prepared. In these wells solvent extracts of the spice were added. The plate was incubated overnight at 27°C. After incubation the zones of inhibition were measured and recorded. Respective solvent controls were also run simultaneously.

Conformity Test with Nutrient Broth:

The extract was diluted up to 1:100 concentration and three test tubes were prepared:

- 1) Extract+Nutrient Broth+Bacterial Colony
- 2) Extract+Nutrient Broth
- 3) Nutrient Broth+Bacterial Colony

After preparation of the test tubes the samples were kept for 24hrs incubation. Absorbance at 540 nm was measured and the growth in each test tube was compared.

Antibacterial Activity:

Antibacterial activity for the sample Cumin is as follows:

Table 2. Zone of inhibition of the extract showing antibacterial activity

BACTERIA	SOLVENT	ZONE OF INHIBITION
<i>Escherichia coli</i>	Acetone (50%)	10mm
	Acetone (100%)	15mm
<i>Staphylococcus aureus</i>	Acetone(50%)	12mm
	Acetone (100%)	16mm

Antifungal Activity:

The antifungal activity of Cumin is as follows:

Table 3. Zone of Inhibition showing antifungal activity

FUNGUS	SOLVENT	ZONE OF INHIBITION
<i>Aspergillus niger</i>	Acetone (50%)	No zone
	Acetone (100%)	No zone
<i>Penicillium notatum</i>	Acetone (50%)	No zone
	Acetone (100%)	No zone
<i>Trichoderma viride</i>	Acetone (50%)	No zone
	Acetone (100%)	No zone
<i>Mucor indicus</i>	Acetone (50%)	No zone
	Acetone (100%)	No zone

Confirmatory Test for bacteria

Table 4. Confirmatory test using Nutrient Broth

BACTERIA	SOLVENT	ABSORBANCE			
		A	S	X	C
<i>Escherichia coli</i>	Acetone (50%)	0.31	0.29	0.03	0.46

Where A stands for All contains sample, nutrient broth and *E.coli*; S for nutrient broth and sample; X is A-S and C for control (Nutrient broth & *E.coli*).

Discussion

The overall evaluation of this study shows that the cumin extract has a good antimicrobial potential. The volatile components of the extract of spices showed appreciable amounts of antibacterial activity against Gram positive bacteria, Gram negative bacteria with prominent zone of inhibition. 100% acetone extracts were found to have better antimicrobial action than any other solvents like ethanol, nhexane, 50% acetone extracts. So this study concludes that cumin have good antioxidant potential and this spices can be used to produce novel natural antioxidants as well as flavoring agents that can be used in various food products. The oils of cumin seed extract showed greater antibacterial activity against all strains tested, with a prominent zone of inhibition. Especially for *E.coli*, the extract showed maximum result which is similar to the result reported by Nanasombat & Lohasupthawee (2005). The concentration of extracting solvent significantly affected contents of cumin seeds extract. The optimal extraction solvent yielding the highest content of non-volatile components in cumin extract is

100% acetone which is also similar to result reported by Bettaieb *et al.* (2010).

Conclusion

Cumin seed extract can be utilized as an anti-bacterial agent to kill gram positive & gram negative bacteria such as *Staphylococcus aureus* and *Escherichia coli* in-vitro. Further investigations on identification and separation of antimicrobial components (chemicals) from cumin seed will be done along with efficiency testing. Comparative studies will be carried out on different fungal species using different extraction methods with different solvents. This type of study will lead us to advancement of medical sciences along with a complete knowledge on naturally occurring antimicrobial components from cumin seed & their applications.

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