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Antimicrobial and Anti-Diabetic Effect of Cumin Seed Extract

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Abstract: Cumin (*Cuminum cyminum*) is a small herbaceous plant that is a member of the aromatic plant family. Cumin is important source of energy, strengthens immune system against many diseases. Medicinal properties of cumin seeds including Immunomoulatory activities as well as Anti- inflammatory, Antimicrobial, Antioxidative effects and very effective in control of diabetics. Cumin is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. The small boat shaped seeds of cumin (*Cuminum cyminum*) have been used for many medicinal and culinary purposes from the ancient time in the various countries. The cumin seeds were collected from Vadapalani market, Chennai, Tamil Nadu, India. The dried sample was pulverized using pestle and mortar. The air dried cumin powders were extracted in a soxhlet extract apparatus with ethanol for preparation of crude ethanol extract. Screening of phytochemical analysis of cumin seeds revealed the presence of saponins, flavonoids, protein and phenol. The antimicrobial activity of cumin seed extracts showed maximum efficiency against *Streptococcus* sp., at 1000 µg/ml and *Aspergillus niger* at 1000 µg/ml and the anti-diabetic activity studied by *in vitro* assay by MTT in RIN5F cell line.

Key words: Cumin Seed Extract · Antibacterial · Antifungal and Anti-diabetic Activity

INTRODUCTION

Medicinal plants are the richest bio for their medicinal drugs [1]. Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants due to the ancient belief which says plants are created to supply man with food, medicinal treatment and other effects [2]. Treatment with medicinal plants is considered very safe as there is no or minimal side effect, use of herbal treatment is independent of any age groups [3]. Spices are sometimes used in medicine, cosmetics or perfume production and applied to treat infectious diseases against pathogenic bacteria and fungi [4].

Cumin is a small herbaceous plant that is a member of the aromatic plant family. It is a native of the Eastern Mediterranean countries and upper Egypt but is now cultivated in Morocco, Iran, Turkey, India, China and America [5]. The spice has a peculiar, strong odor and the flavor is warm and slightly bitter and cumin oils used in aromatherapy [4]. Medicinal properties of cumin seeds including immuno-modulatory activities as well as anti- inflammatory, antimicrobial, antioxidative effects and no toxic effects of cumin seeds were observed in animal models or no serious side effects were observed in clinical trials [3]. Cumin oils and cuminic aldehyde were effective against different Gram positive and Gram negative bacteria isolated from different sources of food and clinical isolates [6]. Oral administration of cumin for six weeks to diabetic rats resulted in significant reduction in blood glucose and body weight. Cumin supplements were found to be more effective than glibenclamide in the treatment of diabetes mellitus. In a glucose tolerance test conducted in rabbits, cumin significantly increased the area under the glucose tolerance curve and hyperglycaemia peak [5].

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MATERIALS AND METHODS

Collection of Sample: The cumin seeds were purchased from the Commercial market at Vadapalani, Chennai, Tamil Nadu, India. The sample was transported to the laboratory immediately for analyses. The sample was cleaned and air dried for one day to remove moisture if any. The dried sample was pulverized using mortar and pestle.

Preparation of Extract: The air dried powdered cumin seeds were extracted in a continuous extraction apparatus (Soxhlet) until exhaustion with ethanol for preparation of crude ethanol extract. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40°C. Crude extract of cumin was kept in deep freeze till used.

Phytochemical Analysis

Test for Tannins: One ml of sample was taken, to that few drops of 0.1 % Ferric chloride was added and observed for brownish Green or Bluish black coloration.

Test for Saponins: One ml of sample was taken, to that 2 ml of water was added. The suspension was shaken in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of Saponins.

Test for Flavonoids: One ml of sample was taken, to that add NaOH, observe Yellow color and Hydrochloric acid concentrated was added and observed for White colour.

Test for Alkaloids: One ml of sample was taken, to that few drops of Drang and of reagent was added. A prominent yellow precipitate indicates the test as positive.

Test for Protein: One ml of sample was taken, to that few drops of Bradford reagent was added. A blue color indicates the presence of Protein.

Test for Steroids: One ml of sample was taken, to those two drops of 10% Concentrated Sulphuric acid was added and observed for Brown colour.

Test for Anthraquinones: One ml of sample was taken, to that aqueous ammonia was added and observed for change in colour. Pink, red, or violet colour in aqueous layer indicates the presence of Anthraquinones.

Test for Phenol: One ml of sample was taken, to that 3 ml of 10 % Lead acetate solution was added a bulky White precipitate indicates the presence of Phenolic compounds.

Antibacterial Activity Assay: The antibacterial activity assay was done by Disc diffusion method on Mueller Hinton agar (MHA) plates against nine bacterial inoculants *viz*., *Streptococcus* sp*., Staphylococcus* sp*., Escherichia coli, Salmonella* sp*., Pseudomonas aeruginosa, Lactobacillus* sp*., Vibrio* sp*., Klebsiella pneumoniae* and *Proteus* sp. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc was placed in MHA plates and add 20 µl of sample (Concentration: 1000 µg, 750 µg and 500 µg) were placed in the disc. The plates were incubated at 37°C for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

Antifungal Activity Assay: The antifungal activity assay was done by Disc diffusion method on Sabouraud Dextrose agar (SDA) medium against four fungal inoculants *viz*., *Aspergillus niger, Microsporum* sp*., Trichopyton* sp. and *Candida* sp. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc was placed in SDA plates and add 20 µl of sample (Concentration: 1000 µg, 750 µg and 500 µg) were placed in the disc. The plates were incubated at 28°C for 24 hrs. Then the antifungal activity was determined by measuring the diameter of zone of inhibition.

Antidiabetic Activity: Cell lines were obtained from National Centre for Cell Sciences, Pune, India (NCCS). The cells were maintained in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10 % FBS, Penicillin (100 U/ml) and Streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C and *in vitro* assay was done by MTT in RIN5F cell line [7].

RESULTS AND DISCUSSION

Cumin seed have been widely used in traditional medicine as diuretic, antihypertensive liver tonic, digestive, antidiarrheal, appetite stimulant, emmenagogue, analgesic, anthelmintic, antibacterial and useful in skin disorders. Consequently, kalonji has been extensively studied for its biological activities and has been shown to

be antidiabetic, anticancer and immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmoltic, bronchodilator, hapatoprotective, antihypertensive, renel protective and antioxidant properties [8].

Cumin has been found to possess various pharmacological activities such as antimicrobial, antidaibetic, antiepileptic, antifertility, anticancer, antioxidant and Immunomodulatory due to the presence of various chemical constituents [5]. The phytochemical analysis of cumin extract revealed the presence of saponins, flavonoids, proteins and phenol. Tannins, alkaloids, steroids and anthroquinones revealed absence in cumin extract (Table 1). Cumin *(Cuminum cyminum*) is an important and popular spice locally known as zeera, contains some important components such as pinene, cymene, terpinene, cuminaldehyde, oleoresin and thymol show their efficacy against various diseases. It is an important source of energy, strengthens immune system and gives protection against many diseases [9]. *Cuminum cyminum* contained alkaloid, coumarin, anthraquinone, flavonoid, glycoside, protein, resin, saponin, tannin and steroid [1].

The antibacterial activity of cumin seeds with ethanol extract at various concentration 1000 µg/ml, 750 µg/ml and 500 µg/ml against organisms were tested (Table 2). The maximum zone of inhibition was showed at *Streptococcus* spp. of 29 mm at 1000 µg/ml. The maximum zone of inhibition was showed at *Pseudomonas aeruginosa* of 24 mm at 750 µg/ml. The maximum zone of inhibition was showed at *Pseudomonas aeruginosa* of 21 mm at 500 µg/ml.

Alkaloids were separated from black seed by using two types of solvents; ethanol and chloroform, we obtained two fractions of alkaloid (A1 and A2). Separated fractions were tested in different concentrations 50, 75, 100, 125 and 150 mg/ml against four types of bacteria (*Staphylococcus aureus*, *Bacillus cereus, Escherichia coli* and *Pseudomonas* spp.). The results showed that both fractions of alkaloid have antibacterial activity against tested bacteria but the effect of A1 was more than A2 and Gram positive bacteria were more sensitive than Gram negative bacteria [10]. Ethanol extracts of seed of cumin were tested for antibacterial activity *in vitro* by the Micro-dilution method [1].

The antifungal activity of cumin seeds with aqueous extract was tested against the organisms with various concentrations of 1000 µg/ml, 750µg/ml and 500 µg/ml (Table 3). The maximum zone of inhibition *Aspergillus niger* of 15 mm was showed at 1000 µg/ml followed by 14 mm of 750 µg/ml and the least zone of inhibition was recorded at 500 µg/ml of 13 mm. Antifungal activity of cumin was recorded against soil, food, animal and human pathogens including dermatophytes, yeast, aflatoxins and mycotoxin producers. Fungal and yeast cultures were more sensitive to cumin volatile oil and cuminaldehyde than Bacteria [5].

The antidiabetic effect of cumin seed were analyzed in cell line culture and *in vitro* assay using MTT in RIN5F cell line and the cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. A volume of 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The Percentage Cell Viability was calculated using the following formula:

% Cell viability = A570 of treated cells/A570 of control cells × 100

Graphs are plotted using the Percentage of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments shown in Table 4 and Figure 1. Diabetes mellitus is a metabolic disease associated with hyperglycemia. DM is estimated to affect about 366 million by 2030. Type II Diabetes has increased during the recent years, its affect 90-95 of diabetic patient. Effective control of hyperglycemia in diabetic patients is critical for reducing the risk of micro and macrovascular complications. Natural sources play an important role in the management of diabetes mellitus, especially in developing countries, delaying the development of diabetic complications and correcting the metabolic abnormalities. Exogenous insulin/oral hypoglycemia drugs can be used to control hyperglycemia in diabetic patient. Sometimes the side effect due to interaction between medicines. So, search for natural alternatives to control diabetes [3].

Table 1: The Phytochemical studies of the sample Cumin seed extract

Test	Sample (Cumin seeds extract)
Tannins	Negative
Saponins	Positive
Flavonoids	Positive
Alkaloids	Negative
Proteins	Positive
Steroids	Negative
Anthroquinones	Negative
Phenol	Positive

Table 2: Antibacterial activity of Cumin seed extract

	Zone of Inhibition (mm)			
Organisms	1000 µg/ml	750 µg/ml	500 µg/ml	Antibiotic (1mg/ml)
Streptococcus sp.	29	16	14	45
Staphylococcus sp.	26	22	19	32
Escherichia coli	10	9	9	27
Salmonella sp.	9	9	8	32
Pseudomonas aeruginosa	27	24	21	31
Lactobacillus sp.	13	12	11	40
Vibrio sp.	9	8	8	30
Klebsiella pneumoniae	10	8	7	25
Proteus sp.	28	19	12	22

Table 3: Antifungal activity of Cumin seed extract

	Zone of Inhibition (mm)			
Organisms	1000 µg/ml	750 µg/ml	500 /ml	Antibiotic (1mg/ml)
Aspergillus niger	15	14	13	16
Candida sp.	10	10	9	11
Microsporum sp.	14	14	12	16
Trichopyton sp.	NZ	NZ	NZ	10

NZ – No zone of inhibition

Table 4: Antidiabetic effect of Cumin seeds *on RIN5f* cell line

S.No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.587	44.06
2	500	1:1	0.653	49.02
3	250	1:2	0.719	53.97
4	125	1:4	0.785	58.93
5	62.5	1:8	0.851	63.88
6	31.2	1:16	0.927	69.59
7	15.6	1:32	1.044	78.37
8	7.8	1:64	1.110	83.33
9	Cell control	1:64	1.332	100

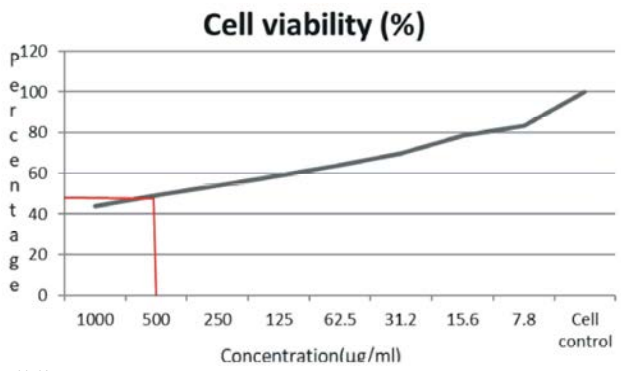

Fig. 1: MTT assay in RIN5-F cell line

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