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Studies on the Antimicrobial Activity of Cumin (*Cuminum Cyminum)* and Fenugreek (*Trigonella Foenum-Graecum*) Extracts Against Certain Food Borne Bacterial Pathogens

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Abstract: *In the present study, extracts of two spices namely cumin and fenugreek solvents were evaluated for their antibacterial and antifungal activity. The antibacterial activity was measured by agar well diffusion method and antifungal activity by poisoned food technique. All the extracts showed antibacterial activity against all the test bacterial isolates. Aqueous extracts of cumin did not exhibit antibacterial activity against B. subtilis. In aqueous extract, cumin and fenugreek showed good inhibitory activity against Staphylococcus aureus with zone of inhibition 20 to 25 mm and 22 to 24 mm respectively. In ethanol extract, cumin extract showed antibacterial activity against all test bacteria with zone of inhibition ranged between 10mm and 18mm, while fenugreek showed activity with zone of inhibition ranged between 10mm and 21mm. In methanol extract, the diameter of zone of inhibition ranged between 9mm and 23mm in cumin and 13mm and 22mm in fenugreek. In case of antifungal activity, only fenugreek ethanol extract showed activity only against Rhizopus stolonifer and Mucor sp. The percent mycelial growth inhibition ranged between 20 to 25%. Based on this finding, these extracts is an alternate to chemical preservatives and can be used as a natural antimicrobial preservative to increase the shelf-life of food.*

Keywords: Agar well diffusion, antibacterial and antifungal activity, cumin, fenugreek extracts

I. INTRODUCTION

Plant derived products such as spices, fruit preparations, vegetable preparations or extracts have been used for centuries in the preservation and extension of the shelf life of foods [1]. Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves (coriander, mint), flower (clove), bulbs (garlic, onion), fruits (red chilli, cumin), stem (cinnamon), rhizomes (ginger, fenugreek) and other seeds.

Prevention of pathogenic and spoilage microorganisms in food is usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic attributes as well as residual toxicity and with growing concern of microbial resistance towards conventional preservatives, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobial for food preservations receives increasing attention [2]. The objective of this study was to evaluate the antimicrobial activity of two plant extracts against food associated bacteria and fungi.

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II. MATERIALS AND METHODS

Evaluation of Antimicrobial Activity of Seed Extracts

Collection of Seeds

Seeds of cumin (*Cuminum cyminum*) and fenugreek (*Trigonella foenum-graecum*) were collected and evaluated for their antimicrobial activity against six food associated bacteria and four fungi. These seeds were selected on the basis of their use in folk medicine and as alternative system of health care and as food preservatives.

Isolation and Identification of Bacteria

Selective media such as Mac Conkey, and Tryptic soy media were used for the isolation of *E. coli* and *Staphylococcus*. Identification was carried out on the basis of morphological and biochemical characteristic through gram staining, catalase test, indole production test, methyl red test, voges - proskauer test, starch hydrolysis and sugar fermentation.

Test Microorganisms

Seven food-associated bacteria (4 Gram-positive and 3 Gram-negative) isolates (*Bacillus subtilis, B. megaterium, Staphylococcus pneumoniae, Staphylococcus aureus*, (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative) and four molds (*Aspergillus luchuensis*, *A. flavus*, *Penicillium oxalicum, Rhizopus stolonifera, Mucor* sp.) were isolated and identified from different food products and screened against plant extracts.

Determination of Bacterial Growth

The isolates were inoculated in the broth and incubated in the shaking incubator at 120 rpm and then the optical density were determined at A640 nm for every 2 hrs.

Protocol for Phytochemical Extraction

For extraction, seeds were thoroughly washed with tap water followed by sterile distilled water. The seeds was dried in an oven at 50°C for 48 hrs followed by grinding in to a fine powder. The powdered material was stored in air tight jars in refrigerator at 4°C [3]. Three extractants i.e., water, ethanol (95%) and methanol (95%) were used for the phytochemical extraction. A total of 3 extracts (aqueous, ethanol and methanol) were prepared from seeds.

For extraction with water, 25 g of powdered seeds was dissolved in enough sterilized distilled water to make 100ml of aqueous extract (25% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 25 ml extract was left in the container. For extraction with ethanol, twenty-five gram of powdered seeds was dissolved in enough ethanol to make 100ml of ethanol extract (25% w/v). The extraction procedure followed was the same as used for aqueous extract. For extraction with methanol, twenty-five gram of powdered seeds was dissolved in enough methanol to make 100ml of methanol extract (25% w/v). The extraction procedure followed was the same as used for aqueous extract. Extracts thus obtained were immediately evaluated for antibacterial and antifungal activities (Barreto *et al*., 2002). [4].

Agar well Diffusion Method

The antibacterial activity of 3 crude extracts (aqueous, ethanol and methanol) of 2 seeds against all the seven food-associated bacterial isolates was evaluated by using agar well diffusion method [5,6] (Ahmad and Beg, 2001; Srinivasan *et al*., 2001). Nutrient agar plates were inoculated with 0.1ml of standardized inoculum ($1.5x10^8$ cfu/ml) of each bacterium (in triplicates) and spread with sterile swabs. Wells of 8 mm size were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. 200µl volume of the plant extract was poured into a well of inoculated plates. Solvent (ethanol/methanol) was used as a negative control which was introduced into a well instead of plant extract. Acetic acid was used as a positive control which was introduced into the well instead of plant extract. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar (Rios *et al*., 1988) [7]. After incubation for 24 hrs at 37°C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters.

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Antibacterial activity was recorded if the zone of inhibition was greater than 8 mm (Hammer *et al*., 1999) [8]. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active (Junior and Zanil, 2000) [9]. The mean and standard deviation of the diameter of inhibition zones were calculated.

Poisoned Food Technique

The antifungal activity of 6 plant extracts was evaluated against food-associated fungi by using poisoned food technique. In poisoned food technique, all the food-associated fungi were inoculated on potato dextrose agar plates in triplicates and incubated for 25°C for 3 to 7 days, to obtain young, actively growing colonies of molds. 100µl of plant extract was mixed with 15ml of cooled (45°C) molten potato dextrose agar medium, poured on to the plates and allowed to solidify at room temperature for thirty minutes. A mycelial disc of 6mm diameter, cut out from the periphery of 3 to 7-day old cultures, was aseptically inoculated into the agar plates containing the plant extract. Potato dextrose agar plates with 100µl of solvent and acetic acid were used as negative and positive control respectively (McCutcheon *et al*., 1994).[10]

The inoculated plates were incubated at 25°C and colony diameter was measured and recorded after 7 days. Percent mycelia growth of inhibition was calculated as given below.

$$\text{Percent mycelia growth inhibition} = \frac{\text{Diameter of fungal colony (mean)in control} - \text{diameter of fungal colony ((mean)with plant extract}}{\text{diameter of fungal colony(mean)in control}} \times 100$$

III. RESULTS AND DISCUSSION

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria. Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for years by many cultures to enhance aroma and flavour (Souza *et al*., 2005).[11] The present research work deals with the antimicrobial evaluation of plants (cumin and fenugreek). Each seed was dried and grinded to a fine powder before subjecting to crude phytochemical extraction. The active phytochemical components would be expected to be more concentrated in dry preparation than in fresh seeds (Romero *et al*., 2005).[12]

Table 1: Morphological Character of *E. coli* and *staphylococcus.*

S. No	Character	*E. coli*	*Staphylococcus*
1	Colour	Pinkish, metallic sheen colony	Yellow halo zone around colonies
2	Cell shape	Bacilli	Cocci
3	Cell arrangement	Single	Irregular cluster
4	Grams reaction	-ve	+ve
5	Catalase test	+ve	+ve
6	Fermentation	Acid & gas	Acid
7	Starch hydrolysis	- ve	-ve
8	Indole	+ ve	-ve
9	Citrate uttilization	- ve	-ve
10	MR	+ ve	+ve
11	VP	- ve	+ve

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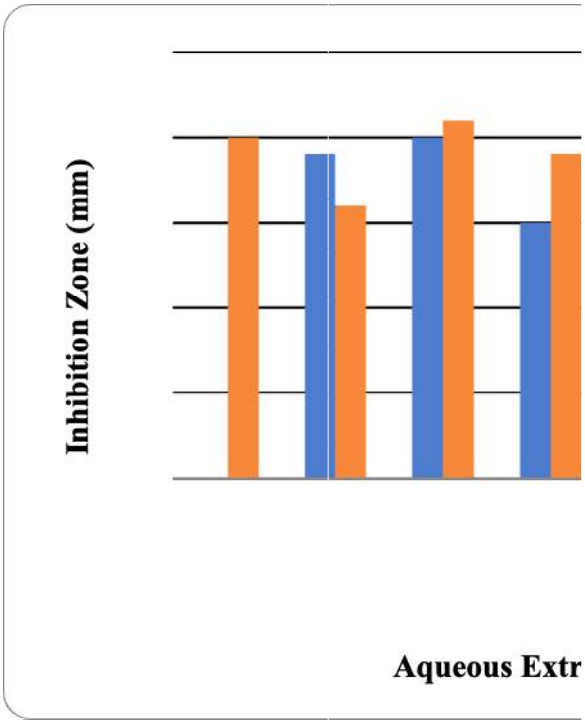
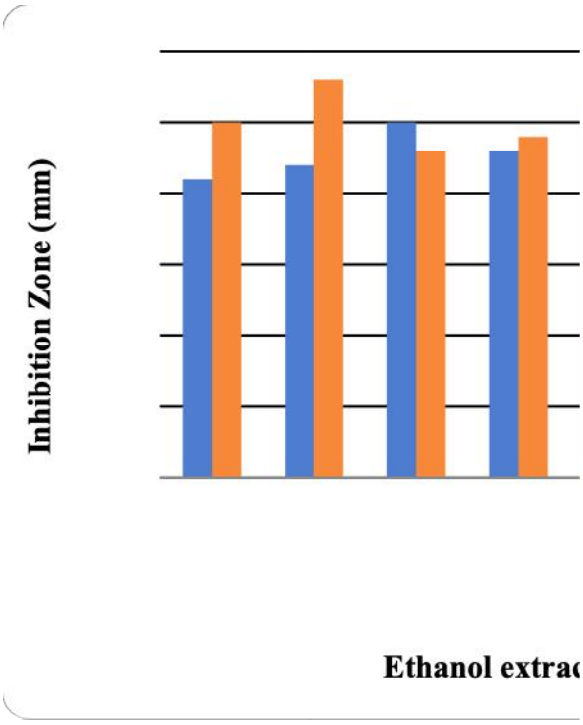
Table 2: Antibacterial activity of two seed extracts in three solvents against food – associated bacteria by agar gel diffusion method





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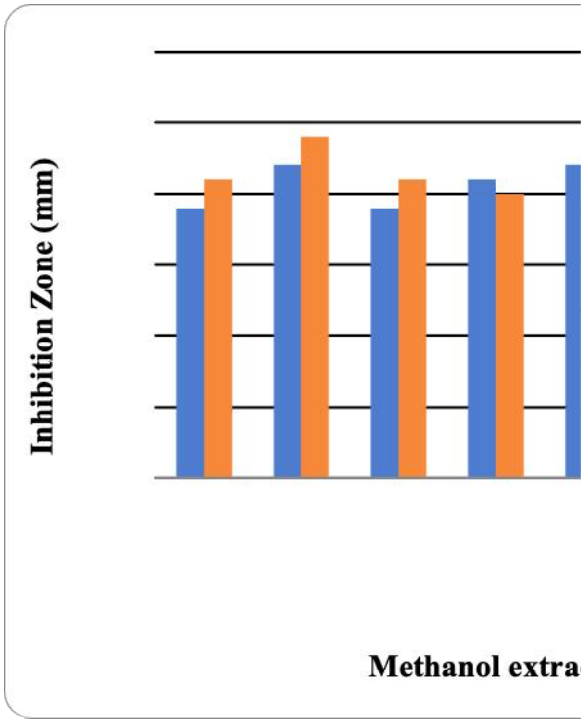

Naturally occurring water-soluble components in most seeds include various anionic components such as thiocynate, nitrate, chlorides and sulphates, starches and tannins, saponins, terpenoids, polypeptides and lectins (Darout *et al*., 2000) [13]. Phytochemicals having solubility in ethanol include tannins, polyphenols, polyacetylenes, flavonol, sterols and alkaloids (Ivanovska *et al*., 1996) [14]. Cowan,[15] examined a variety of extractants for their ability to solubilize anti-bacterials from plants as well as other factors such 5s their relative ranking as biohazards and the ease of removal of solvent from the fraction and ranked them in the order: methylene dichloride > methanol > ethanol > water. Accordingly, in the present study, three solvents namely water, ethanol and methanol were selected for the plant extraction. In the present study, the cumin and fenugreek extracts exhibited antibacterial activity in all the three kinds of solvents.

Aqueous, ethanol and methanol extracts of cumin exhibited activity against *B. megaterium, B. sphaericus, B. polymyxa, S. aureus* and *E. coli*, this substantiate the findings of [16] Ali *et al*., (2007), who had been reported antibacterial activity of water, petroleum ether, ethyl acetate, ethanol and methanol cumin extracts against *B. megaterium, B. subtilis, S. aureus* and *E. coli*. According to Harold (2004) [17], the antimicrobial activity of cumin is due to the presence of essential oil (3%), whose aroma is dominated by monoterpenes hydrocarbons: sabinene, β-pinene and limonene. Furthermore, terpinene, α-pinene, myrcene, and monoterpene derivatives like borneol, carvone, carvacrol, 1, 8-cineol and linalool are also present. The mechanism of action of terpene is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Ahmed *et al*., 1993) [18]. A perusal of the data in the table 2 reveals that all the three types of cumin and fenugreek extracts possessed activity against all the food associated bacteria. The ethanol extract being strongly active against *E. coli* isolates while aqueous extracts strongly active against *S. aureus* isolates (25mm- 30mm).

Fenugreek ethanol extract showed inhibitory activity against only two molds namely *Aspergillus flavus* (10mm) and *Mucor* sp.(15mm). Fenugreek has also been shown to induce antifungal activity.

The fungistatic or fungicidal effect of spices is due to the inhibitory action of natural products and the mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating with mycelium germination inhibition (Cowan, 1999) [15] and it is also reported that plant lytic enzyme acts in the fungal cell wall causing breakage of β-1,3 glycan, β-1,6, glycan and chitin polymer.

These extracts did not possess antifungal activity except fenugreek ethanol extract against the food-associated molds. There may be several seasons for the lack of antimicrobial activity in their plants, either the plant part used or the type

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of extraction might have resulted in the nil activity in this study. The time of collection of herbal material and climate, which might, in turn, affect the amount of active constituents in the seeds (Parekh and Chanda, 2007). [19]

The antimicrobial action of the aqueous extracts could be ascribed to the anionic components such as thiocyanate, nitrate, chlorides and sulphates besides other water-soluble components which are naturally occurring in the seeds (Darout *et al*., 2000). The ethanol extraction of herbs and spices was better because ethanol is an organic solvent and dissolves more organic compounds, resulting in the liberation of the greater amounts of active antimicrobial components (Cowan, 1999).[15] The water was found to be least effective in extracting the active antimicrobial component/s present in the spices in the present study. The differences in the sensitivity of food associated microorganisms may be due to the differences in concentrations, methods of extraction used in each study (Kumar *et al*., 1997) [20] and the little diffusion properties of these extracts in the agar and soil composition and water availability (Romero *et al*., 2005). [12]

IV. CONCLUSION

It is concluded from the present studies that both the extracts can be used as a potential source of natural antimicrobial compound which if applied to food products. Further research is required for the identification of bioactive molecule present in the two extracts and *in vivo* efficacy against food spoilage microorganisms have to be analyzed before commercialization in the form of food preservatives, additives and nutraceutical foods.

The results obtained during this experiment for the three solvent extracts in the order of effectiveness is as follows (aqueous> ethanol> methanol)

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