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ANTIBACTERIAL ACTIVITIES OF *TRIGONELLA FOENUM-GRAECUM* AND *ZINGIBER OFFICINALE*

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ABSTRACT

An *in vitro* study was conducted for screening antibacterial activities of seeds of *Trigonella foenum-graecum* (fenugreek) and rhizomes of *Zingiber officinale* (ginger), in their different forms *viz.* aqueous extracts, essential oils and powders against some common food borne pathogens. Bacterial strains involved in the study were *Bacillus cereus (MTCC 430)*, *Enterococcus feacalis (MTCC 439)*, *Escherichia coli (MTCC 1687), Psuedomonas aeruginosa (MTCC 424)* and *Staphylococcus aureus (MTCC 5021).* Results revealed that powdered forms of *T. foenum-graecum* and *Z. officinale* remained ineffective in arresting the growth of all the bacterial strains under investigation. During preliminary screening, aqueous extracts and essential oils of test spices did not exhibit any growth inhibitory zone towards any test bacteria. In broth dilution technique, essential oil of *Z. officinale* arrested all the bacterial strains under observation while essential oil of *T. foenum-graecum* and aqueous extracts of *T. foenum-graecum* and *Z. officinale* remained ineffective.

INTRODUCTION: Current economic and biological assessment upon withdrawal of most of the conventional synthetic preservatives registered for control of microorganisms implicated in food spoilage have elicited widespread interest in providing new perspectives for the development of future antimicrobials based on natural substances, those are socially more acceptable [1]. According to the council for Agriculture Science and Technology, an international consortium of 36 scientific and professional societies, many biologically derived substances from plant and animal sources exhibit antimicrobial properties in foods in which normally they are found or may be developed for commercial use as preservative to other food products. Biologicals, because of their natural origin, are biodegradable and mostly do not leave toxic residues or byproducts to contaminate the environment and spices are one among them.

T. foenum-graecum (fenugreek) is an important spice in India, Egypt, Saudi Arabia, Iran, Armenia and Turkey. *Trigonella* seeds are bitter, mucilaginous, aromatic, tonic, emollient, anticarcinogenic [2], anti inflammatory [3], hypoglycaemic [4], hepatoprotective [5], immuno-modulatory [6], larvicidal [7] and wound healing activities [8]. These are rich source of phytochemicals, especially the steroidal saponins (chiefly composed of diosgenin), which are the starting compounds for the manufacture of over 60% of the total steroid drugs by the pharmaceutical industry .

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Z. officinale (ginger) is generally consumed as fresh paste and dried powder in culinary practices and for flavoring tea. The pungent compounds of *Z. officinale* include gingerols, shogaols, paradols and zingerone [9,]. Rhizomes of *Z. officinale* and its constituents have been proven for their antioxidant [10] and antiplatelet efficacies [11].

Present study focuses on the growth inhibitory activities of *T. foenum-graecum* and *Z. officinale* towards some common food borne pathogens.

MATERIALS AND METHODS:

Procurement of Spice Samples: Dried seeds of *T. foenum graecum* were procured in a single lot, in the amounts of 500 g, from a wholesaler spice-seller, local market, Hisar, India. Procured seeds were cleaned manually for extraneous material and were ground to powdered form in laboratory grinder. Fresh rhizomes of *Z. officinale* (rhizomes) were purchased in the amounts of 1 kg, from grocery shop, local market, Hisar, India. *Z. officinale* rhizomes were washed with distilled water to remove extraneous matter followed by their peeling and drying in shade for 5 days. Dried rhizomes thus obtained were ground to powdered form. Commercial preparations of essential oils of *T.foenum graecum* and *Z. officinale* were used.

Chemicals and Culture Media: Ethyl violet azide dextrose agar, Ethyl violet azide dextrose broth, MacConkey agar, MacConkey agar, MacConkey broth, Nutrient agar and Nutrient broth were obtained from Hi-Media Pvt. Ltd, India. Dimethylsulphoxide (DMSO) and Sodium chloride (NaCl) were purchaged from Central drug house Pvt. Limited, India.

Bacterial cultures: Pure cultures of *Bacillus cereus (MTCC 430)*, *Enterococcus feacalis (MTCC439)*, *Escherichia coli (MTCC 1687), Psuedomonas aeruginosa (MTCC 424)* and *Staphylococcus aureus (MTCC 5021)* were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference bacterial strains were maintained on respective media slants, subcultured bimonthly to maintain their viability and were stored at 4±1°C. Bacterial strains were adjusted with sterilized normal saline to contain approximately 1×10^7 cfu/ml (used as inoculum for experiments). Incubation temperatures, incubation periods and media used for test microbes are mentioned in **Table 1**.

TABLE 1: BACTERIAL STRAINS TESTED

Bacterial strains	Strain number	Media used	Temperature of incubation	Time period of incubation
B. cereus	MTCC 430	Nutrient agar, Nutrient broth	30^0C	24-48 h.
E. faecalis	MTCC 439	Ethyl violet azide dextrose agar, Ethyl violet azide dextrose broth	45^0C	24-48 h.
E. coli	MTCC 1687	MacConkey agar, MacConkey broth	45^0C	24-48 h.
P. aeruginosa	MTCC 424	Nutrient agar, Nutrient broth	32^0C	24-48h.
S. aureus	MTCC 5021	Nutrient agar, Nutrient broth	37^0C	24-48 h.

Prepearation of Aqueous Spice Extracts: Aqueous extracts were prepared in the laboratory [12]. Powdered spice samples of *T. foenum-graecum* and *Z. officinale* were steeped overnight (temperature: 24-27°C) in sterilized distilled water in a ratio of 1:1 (w:v), followed by their homogenization in a blender at high speed for 2 min. The homogenized spice mixtures were filtered through Whatman No. 1 filter paper. Filtrates thus obtained, were sterilized by passing through syringe filters containing 0.45 um pore size membrane filters and were collected in sterilized glass vials.

Determination of antibacterial activities of test spice samples: Spice agar method [13] was opted for investigating antibacterial activities of powdered forms of test spices at their different concentration levels (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0%, (w/v)). The petriplates supplemented with abovestated spice concentrations were examined for bacterial growth at an interval of 12 hours, for an incubation period of 30 days.

Similar sets of experiments were conducted without any spice sample that served as negative control. Agar well assay [14] was used for the preliminary screening of aqueous extracts (50, 80, 100 (ul/well)) and essential oils (30ul/well) of reference spices against bacterial strains under observation. Dimethylsulphoxide (DMSO) served as negative control.

Results were expressed as net zones of inhibition. Broth dilution technique [15] was followed for the better insight into the antibacterial activities of test spice samples (aqueous extacts and essential oils). Test substances were used at various concentration levels *viz.* 2000, 1000, 500, 250, 125, 62.50, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97, 0.48, 0.24, 0.12, 0.06 ul/ml; and the concentrations, at which bacterial growth was fully inhibited, were recorded. Dimethylsulphoxide (DMSO) served as negative control. All the abovementioned experiments were conducted in triplicates results obtained were highly reproducible.

RESULTS AND DISCUSSION: The results revealed that powdered forms of *T. foenum-graecum* and *Z. officinale* upto 6.0% level (w/v), remained ineffective in arresting the growth of bacterial strains under observation, and visible growth of all the bacterial strains was noticed on 2nd day of incubation as in control set of petriplates containing no spice sample (**Table 2**). The potential of spices in the culinary, non culinary and medicinal fields is based on the chemistry and composition of their volatile aromatic secretions generally known as essential oils. Seeds of *T. foenum-graecum* contain 0.02-0.05% essential oil [16], while rhizomes of *Z. officinale* are composed of 2-3 % essential oil [17]. The inertness of powdered forms may be attributed to very high volatility and subsequent losses of antimicrobial components during grinding and drying. Composition of fresh and dried rhizomes of *Z. officinale* was determined by Gas chromatography (GC) and GC-MS techniques and it was observed that fresh rhizomes usually contain a greater proportion of the lower boiling components and up to 80% of the volatile components can be lost during drying [18]. During preliminary screening aqueous extracts (50, 80, 100 (ul/well)) and essential oils (30 ul/well) of both the test spices did not exhibit any growth inhibitory zone towards any reference bacterial strain (**Table 3**).

However, from the results of broth dilution technique, it was found that essential oil of *Z. officinale* at different concentrations *viz.* 250 (ul/ml), 1000 (ul/ml), 2000 (ul/ml), 1000 (ul/ml), 250 (ul/ml) inhibited *B. cereus, E. faecalis, E. coli, P. aeruginosa, S. aureus* respectively, while essential oil of *T. foenum-graecum* and aqueous extracts of *T. foenum-graecum* and *Z . officinale* could not arrest test bacteria (**Table 4**). It was also noticed that g+ve bacterial strains were inhibited at lower concentration levels of essential oil of *Z. officinale.* This differential behaviour of test microbes may be attributed to their different membrane structures and their relative permeability towards substrate components [19, 20]. The sensitivity of microbes towards essential oil of *Z. officinale* followed the following order : *B. cereus = S. aureus > E. faecalis = P. aeruginosa > E.coli.*

TABLE 2: INHIBITORY EFFECTS OF POWDERED FORMS OF *T. FOENUM-GRAECUM* AND *Z. OFFICINALE* ON THE GROWTH OF BACTERIAL STRAINS

Spice Conc. (%,w/v)	Days of inhibition									
	B. cereus		*E. faecalis*		*E. coli*		*P. aeruginosa*		*S. aureus*	
	T. f-g.	*Z.o.*	*T. f-g.*	*Z.o.*	*T. f-g.*	*Z.o.*	*T. f-g.*	*Z.o.*	*T. f-g.*	*Z.o.*
0.0	2	2	2	2	2	2	2	2	2	2
0.1	2	2	2	2	2	2	2	2	2	2
0.2	2	2	2	2	2	2	2	2	2	2
0.4	2	2	2	2	2	2	2	2	2	2
0.6	2	2	2	2	2	2	2	2	2	2
0.8	2	2	2	2	2	2	2	2	2	2
1.0	2	2	2	2	2	2	2	2	2	2
1.5	2	2	2	2	2	2	2	2	2	2
2.0	2	2	2	2	2	2	2	2	2	2
2.5	2	2	2	2	2	2	2	2	2	2
3.0	2	2	2	2	2	2	2	2	2	2
3.5	2	2	2	2	2	2	2	2	2	2
4.0	2	2	2	2	2	2	2	2	2	2
4.5	2	2	2	2	2	2	2	2	2	2
5.0	2	2	2	2	2	2	2	2	2	2
5.5	2	2	2	2	2	2	2	2	2	2
6.0	2	2	2	2	2	2	2	2	2	2

T. f-g : Trigonella foenum-graecum; Z. o.: Zingiber officinale

TABLE 3: INHIBITORY EFFECTS OF AQUEOUS EXTRACTS AND ESSENTIAL OILS OF REFERENCE SPICES ON BACTERIAL STRAINS (AGAR-WELL ASSAY)

Bacterial strains	Zones of Inhibition (mm)								
		Essential oils (30 ul)		Aqueous extracts					
				(50 ul)		(80 ul)		(100 ul)/'][=	
		T.f.-g.	*Z.o.*	*T.f.-g.*	*Z.o.*	*T.f.-g.*	*Z.o.*	*T.f.-g.*	*Z.o.*
B.cereus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. faecalis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E. coli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S. aureus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

T.f-g : Trigonella foenum-graecum, Z.o.: Zingiber officinale, DMSO: Dimethylsulphoxide

TABLE 4: INHIBITORY EFFECTS OF AQUEOUS EXTRACTS AND ESSENTIAL OILS OF REFERENCE SPICES ON BACTERIAL STRAINS (BROTH DILUTION TECHNIQUE)

Test substances	Spices	Bacterial strains				
		B.cereus	***E.faecalis***	***E.coli***	***P.aeruginosa***	***S.aureus***
Aqueous extract (ul/ml)	*T.f.-g.*	ND	ND	ND	ND	ND
	Z.o.	ND	ND	ND	ND	ND
Essential oil (ul/ml)	*T.f.-g.*	ND	ND	ND	ND	ND
	Z.o.	250.00	1000.00	2000.00	1000.00	250.00
DMSO (ul/ml)	-	ND	ND	ND	ND	ND

T.f-g : Trigonella foenum-graecum, Z.o.: Zingiber officinale, DMSO: Dimethylsulphoxide, ND: Not Detected

CONCLUSION: The results of this *in vitro* study indicate that among all the spice forms tested, only essential oil of *Zingiber officinale* arrested food borne pathogens effectively and hence, it may be considered for further studies (*in vitro* and *in vivo*), against other microbes of spoilage and health significance.

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