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HPTLC Fingerprinting Analysis and Antibacterial Activity of Various Extracts from Fruits of *Cuminum cyminum*

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Abstract

The present study was conducted to evaluate the antibacterial activity of fruit extracts of *Cuminum cyminum*, a spice used throughout the world. Different solvent extracts, prepared, were evaluated for their antibacterial potential by agar-well diffusion assay against bacterial species of clinical significance. MIC values were determined further to check the concentration ranges for significant inhibition. HPTLC fingerprinting analysis was done to separate the components of active crude extract in an attempt to identify the bio-active chemical entity. Methanol extract exhibited more pronounced activity than that of ethanol and aqueous extracts. Excellent inhibitory effect was found against *Staphylococcus aureus* and *Escherichia coli* and their different pathogenic isolates. *Staphylococcus aureus* required about 128-32 μg/ml of the crude methanol extract for effective inhibition while it was recorded 256-128 μg/ml against *E. coli*. HPTLC evaluation at λ 254 nm in mobile phase toluene : ethyl acetate (9.2 : 0.8) was performed for the separation of a complex mixture of the methanol extract. The results provided evidence that *C. cyminum* extracts might indeed be potential sources of new antibacterial agents.

Keywords: *Spices, Drug –resistance, Cuminum cyminum, Methanolic extract, HPTLC fingerprinting*

Introduction

The spices are products of tropical and subtropical lianas, trees, shrubs and herbs characterized by highly pungent odours or flavours. The spices have a unique aroma and flavour which are derived from compounds known as phytochemicals or secondary metabolites (Avato *et al.*, 2000). The phytochemicals are antimicrobial substances present in the spices which are capable of attracting benefits and repel harmful organisms; they also serve as photoprotectants and respond to environmental changes. Numerous classes of phytochemicals including the isoflavones, anthocyanins and flavonoids are found associated with the spices (Capecka *et al*., 2005, Jones *et al*., 1992). The search for medicinal spices with antimicrobial activity has gained importance in recent years due to growing worldwide concern about an alarming increase in the emergence of antibiotic resistance and further increase in the rate of infection by these antibiotic-resistant microorganisms. Many spices are known to exhibit diverse biological activities such as antibacterial and antifungal (Michael Derrida, 1999, Keskin *et al*., 2010, Santoyo *et al*., 2006 and Patumaraj 2000). The aim of study reported here was to investigate the antibacterial activity of various organic solvents extracts of *C. cyminum* against the bacteria of clinical significance.

Materials and Methods

Collection of spice

The ripened fruits of *Cuminum cyminum* were bought from the local grocery store of Haridwar in middle of 2009, and were taken to the laboratory and were identified with the help of taxonomic literature, standard flora and herbarium at Gurukul Kangri University, Haridwar, India.

Aqueous Extract Preparation

50g of powdered fruits were mixed well in 500 ml distilled water with constant stirring for 30 minutes. The solution was kept at room temperature for at least 24 h and then filtered using muslin cloth. The supernatant was again filtered using Whatman's Filter No. 2 under strict aseptic conditions. The filtrate was collected in fresh sterilized glass tubes and stored at 4°C until use. Aqueous extract was prepared in final concentration of 100 mg/ml.

Soxhelt Solvent Extraction

Air- dried powder (50 g) of the respective spice was thoroughly mixed with 500 ml organic solvent (viz.; methanol and ethanol) and subjected to extraction in the Soxhelt apparatus and for 48 h. Thereafter, the extract was further evaporated to dryness at 40°C in a rotavapour under vacuum following the methodology adapted by Tonk *et al*., (2006). When all the solvent was evaporated, the extract was collected in a vial and kept at 4°C in the refrigerator for storage. Stock solutions of the crude extracts from each type of organic solvent were prepared by mixing well the appropriate amount of dried extract with respective solvent to obtain a final concentration of 100 mg/ml. Extracts were stored in glass vials at 4°C for further use.

Bacterial Strains

Two bacterial strains namely; *Staphylococcus aureus* MTCC-740, *Escherichia coli* MTCC-723, were selected for the present study. All strains were collected from the Microbial Type Culture Collection (MTCC), India. The bacterial cultures were maintained in nutrient agar slants at 37°C. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C. The local isolates of same bacterial species were isolated from different water and clinical samples were identified using morphological characteristics, Gram staining and different biochemical tests. *S. aureus* isolated from pus sample was denoted as SaP and that from water sample, SaW, and *E. coli* isolated from urine sample was marked, EcU and that from water, as EcW.

Antibacterial Susceptibility Assay

According to Perez *et al*., (1990) agar well diffusion assay was the key process used to evaluate the antibacterial potential of the extracts. 25 ml of melted Mueller Hinton Agar (MHA) at 40°C was poured into 90 mm glass Petri plates. Agar plates were then cooled to room temperature for solidification. Bacterial lawn ((inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 CFU/ml) was prepared with the help of swab and inoculated plates were left to rest for five minutes. Wells of 3 mm in diameter were cut from the agar with the help of sterilized cup-borer. The plates were turned upside down and marked for the microorganism inoculated; similarly, each well was also labeled for the respective spice extract. 50 µl of respective spice extract was dispensed into suitably labeled wells using a micropipette with sterilized tip. The plates were then incubated at 37±2°C in an incubator for 24 hours. The experiment was performed in triplicate under strict aseptic conditions to ensure consistency of all findings. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period. Organic solvents used in preparation of extracts were also used as negative controls during the study. Comparative analysis of the effectiveness of Ciprofloxacin (5µg/ml) as a standard antibiotic (positive control) and various solvents used for the test spice extracts against test organism were also carried out.

Assessment of Minimum Inhibitory Concentration

MIC (minimum inhibitory concentration) of active extracts so obtained was further examined by standard two-fold microdilution broth methodology NCCLS (1997). A stock solution of each active extract was serially diluted in 96-well microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 1.0 µg/ml to 512 µg/ml. A standardized inoculum for bacterial strain was prepared so as to give an inoculum size of approximately 5 x 10^5 CFU/ml in each well. Microtiter plates were then kept at 37±2°C for an overnight incubation. Following incubation, the MIC was calculated as the visible lowest concentration of the extract inhibiting growth of bacterial strain.

HPTLC Fingerprinting and Phytochemical Analysis

HPTLC analysis was performed on a silica gel F- $_{254}$ (E-Merck grade) pre-coated aluminium plate. A band of 7 mm was applied at a distance of 10 mm from the bottom of the plate. The methanol extract (10 µl) was applied on a chromatoplate (CAMAG Linomat-5) and run in the solvent system (toluene : ethyl acetate (9.2 : 0.8)). The spray reagent used was anisaldehyde-sulphuric acid. The plate was developed up to 8 cm in a twin trough chamber previously equilibrated with mobile phase for 20 min. Densitometric evaluation of the plate was performed at λ 254 nm. Active extracts were again analysed for its phytochemistry for presence of alkaloid, flavonoids, terpenaoids and tannin.

Statistical Analysis

Statistical analysis of the data was carried out with analysis of variance (one way ANOVA). One-way ANOVA test was performed using "**Richard Lowry's web based ANOVA calculator**".

Results

The results shown in table 1 and figure1 indicate that the methanol extracts produced a prominent effect on various bacterial species. Zone of inhibition varied between 29.0 mm to 20.0 mm. However, ethanol and aqueous extract exhibited a moderate effect and negative control (methanol) did not exhibit any antibacterial activity. Ethanol extract also showed good activity and its effective zone of inhibition varied between 19.0 mm to 7.0 mm but this was found resistant against EcU, SaW and SaP. While aqueous extract showed very less activity in range of 12.0 mm to 7.0 mm and considered resistant against all the strains. As shown in table 2 methanol extract of *Cuminum cyminum* was found significantly inhibitory against SaI with MIC of 128 µg ml^{-1}. The same extract was found active against SaW and SaP at 128 µg ml^{-1} and 32 $^{\mu}$g ml^{-1}, respectively. MIC of this extract was recorded against EcI 256 µg ml^{-1}. While 128 µg ml^{-1} and 128 µg ml^{-1} for EcW and EcU, respectively. In the same manner when ethanol extract was tested against SaI and EcI MIC was recorded 128 µg ml^{-1} and 32 µg ml^{-1} for EcW respectively. Results of HPTLC analysis revealed that the methanol extract contained a mixture of different component that were eluted at R_f =0.12, R_f =0.16, R_f =0.18, R_f =0.22, R_f =0.24, R_f =0.26, R_f =0.28, R_f =0.30, R_f =0.33, R_f =0.43, =, R_f =0.42, R_f =0.42, R_f =0.54, R_f =0.55, R_f =0.66, R_f =0.65, R_f =0.74, R_f =0.77 as shown in figure 2. Phytochemical evaluation reveals the presence of alkaloids, flavanoids, tannins and steroids in the methanol extract of *Cuminum cyminum*.

Table 1. Comparative analysis of different extracts of *Cuminum cyminum* against all bacterial isolates

Organism	Methanol Extract			Ethanol Extract			Aqueous Extract		
	ZOI	Std err	P value	ZOI	Std err	P value	ZOI	Std err	P value
SaI(MTCC)	20±2	1.1547	M1vsM2 P>0.05	19±1	0.5774	M1vsM3 P<0.01	8±2	1.1547	M2vsM3 P<0.01
SaW	27±3	1.7321	M1vsM2 P<0.01	12±2	1.5774	M1vsM3 P<0.01	7±1.5	0.866	M1vsM3 P<0.05
SaP	22±2	1.1547	M1vsM2 P<0.05	7±1.5	0.866	M1vsM3 P<0.01	12±1	0.5774	M1vsM3 P<0.05
EcI(MTCC)	29±2	1.1547	M1vsM2 P<0.01	19±1	0.5774	M1vsM3 P<0.01	12±1.5	0.866	M1vsM3 P<0.01
EcW	21±2.64	1.5275	M1vsM2 P>0.05	18.6±.57	0.3333	M1vsM3 P<0.01	7.6±.57	0.3333	M1vsM3 P<0.01
EcU	27±2.5	1.4434	M1vsM2 P<0.01	12±2	0.5774	M1vsM3 P<0.01	12±2	.5774	M1vsM3 P>0.01

Values of observed effective zone of inhibition (ZOI), (in mm diameter) excluding the diameter of the well (3 mm) after 24 h. incubation against different bacterial species when subjected to different extracts in agar well diffusion assay. Assay was performed in triplicate and results are the mean of three values ±Standard deviation. In each well, the sample size was 50 µl. inhibitions observed in extracts due to solvent were assessed through negative control. 'NI'-No inhibition was observed. HSD= the absolute [unsigned] difference between any two sample means required for significance at the designated level. HSD[.05] for the .05 level; HSD[.01] for the .01 level. M1= mean of methanol spice extract, M2= mean of ethanol spice extract and M3= mean of aqueous spice extract, Std err- standard error

Table 2: Minimum Inhibitory Concentrate of Active extract of *Cuminum cyminum*

Test organism	*C. cyminum* Solvent Extracts	Concentration of extracts in µg/ml										MIC
		512	256	128	64	32	16	8	4	2	1	
SaI(MTCC)	Methanol	-	-	-	+	+	+	+	+	+	+	128
	Ethanol	-	-	-	+	+	+	+	+	+	+	128
SaW	Methanol	-	-	-	+	+	+	+	+	+	+	128
	Ethanol	-	+	+	+	+	+	+	+	+	+	512
SaP	Methanol	-	-	-	-	-	+	+	+	+	+	32
	Ethanol	-	-	-	-	+	+	+	+	+	+	64
EcI(MTCC)	Methanol	-	+	+	+	+	+	+	+	+	+	256
	Ethanol	-	-	-	+	+	+	+	+	+	+	128
EcW	Methanol	-	-	-	+	+	+	+	+	+	+	128
	Ethanol	-	-	-	-	-	+	+	+	+	+	32
EcU	Methanol		-	-	+	+	+	+	+	+	+	128
	Ethanol	-	-	-	-	-	+	+	+	+	+	32
	Ciproflaxacin	-	-	-	-	-	-	-	-	-	-	≤1

Note: Different concentrations of active crude extracts evaluated in 96-well microtiter plate using Microbroth Dilution Assay as recommended by NCCLS. All values are expressed in µg ml^{-1}; (-) represents 'No Growth Observed'; (+) represents 'Growth Observed'.

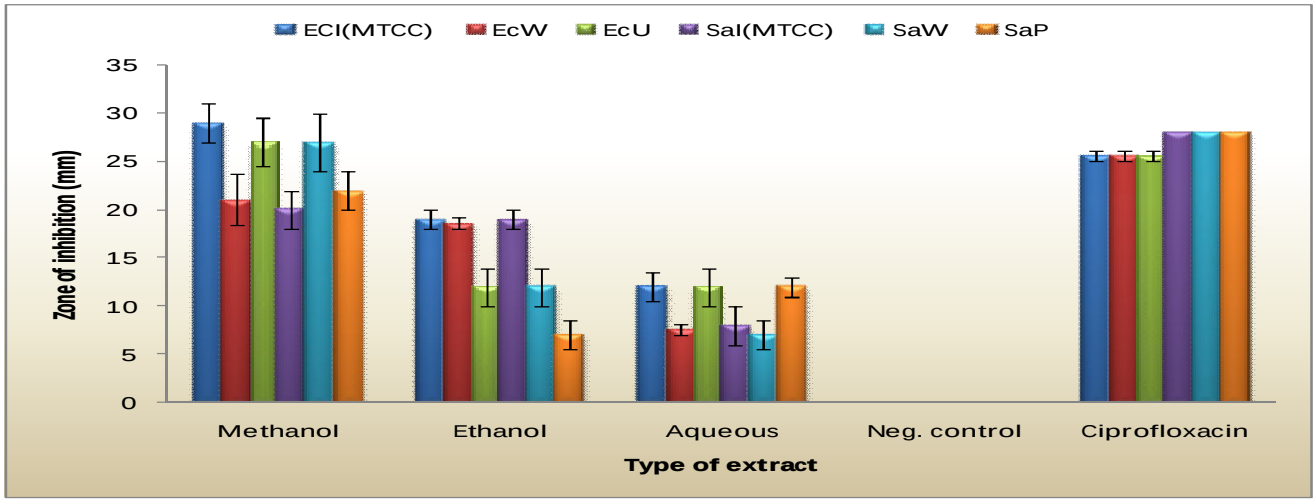
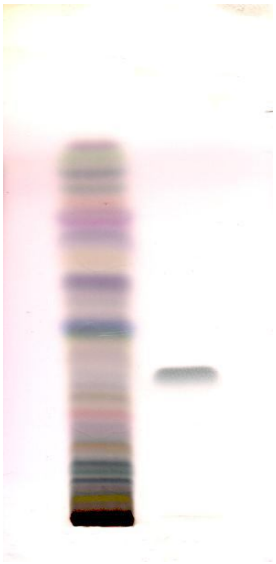


Fig.1 Comparative analysis of different extracts of spice *Cuminum cyminum* against all bacterial isolates



Mobile phase: Toluene : ethyl acetate (9.2 : 0.8)

Fig. 2. HPTLC fingerprinting profile of methanolic extract of *Cuminum cyminum* (observation under 254 nm)

Discussion

There is a clear indication that different solvents extracts of *C. cyminum* produced significant antibacterial effects on both Gram-positive and Gram-negative bacterial species. Akgul and Kivanc, (1989) reported that cumin exhibited an inhibitory effect against *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. Con *et al*., (1998) demonstrated that cumin had an inhibitory effect against *S. aureus* and *M. luteus*. It is interesting to note that Icobellis *et al*., (2005) evaluated the inhibition of different Gram positive and Gram negative bacterial pathogens by essential oil of *Cuminum cyminum*. The current scenario of antibiotics is very threatening due to significant emergence of resistance among bacterial pathogens against available antibiotics. Thus spices can also be a possible source for new potent drugs against pathogenic multidrug resistant bacteria (Fabricant and Farnsworth, 2001). Present study was also supported by Shrinivas *et al*., (2004) to which they attributed to the presence of alkaloid and flavanoid in the fruits of *Cuminum cyminum*. He quantified the flavonoid luteolin from cumin fruits. In another study Sarika *et al*., (2008) revealed the presence of flavanoid, saponins and steroids and also analyzed the flavonoidal glycosidal content by high performance thin

layer chromatographic (HPTLC) analysis using luteolin glycoside as reference compound. The present study focused to the discovery of novel chemicals that can lead to the development of pharmaceuticals of medicinal plants/spices. The combination of a chromatographic technique with serial dilution in tubes was capable of identifying the active components of methanol extract responsible for this activity. This manifests the importance of using the correct combination of chemical Screening and biological analysis.

Conclusion

Present *in vitro* study demonstrated that the spice extract of *Cuminum cyminum* could be a source of new antibacterial compounds. Further studies regarding isolation, purification and evaluation of the compounds from the extracts in order to test specific antimicrobial activity may be helpful to produce an economical and safe alternative to treat the various enteric, skin and urinary infections.

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References

Akgul, A. and Kivanc, M. 1989.Antibacterial effects of spices, sorbic acid, and sodium chloride Doga. Turk. J. Agric, 13: 1-9.

Avato, P., Tursil, E., Vitali, C., Miccolis, V. and Caddido, V. 2000. Allyl sulfide constituents of garlic volatile oil as antimicrobial agents. Phytomedicine, 7: 239-243.

Capecka, E., Mareczek, A. and Leja, M**.** 2005. Antioxidant activity of fresh and dry herbs of some *Lamiaceae* species. Food Chemistry. 93: 223−226.

Con, A.H., Ayar, A. and Gokalp, H.Y. 1998. Antimicrobial activity of the essential oils extracted from some spices. Food, 23: 171-175.

Fabricant, D.S. and Farnsworth, N.R. 2001. The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives Supplements, 109: 69-75.

Iacobellis, N.S., Contare, P.L., Capasso, F. and Senatore, F. 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J Aqric

Food Chem, 53(1): 57-61. Jones, D.P., Coates, R.J., Flagg, E.W., Eley, J.W., Block, G., Greenberg, R.S.,

Gunter, E.W. and Jackson, B. 1992. Glutathione in foods listed in the National Cancer Institute's Health Habits and History Food Frequency Questionnaire. Nutrition and Cancer. 17(1) :57–75.

Keskin, D., Oskay, D. and Oskay, M. 2010. Antimicrobial Activity of Selected Plant Spices Marketed in the West Anatolia. International Journal of Agriculture and Biology, 12 (6): 916–920.

Michael, D. 1999. Common spices protect bacteria during Irradiation. Am. Chem. Soc, 2: 270-275.
NCCLS-National Committee for Clinical Laboratory Standards: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 1997 Approved Standards M7-A4, Wayne, Pa.

Patmaraj, S. 2000. Drug delivery. J. Nucl. Med., 7: 91-95.
Perez, C., Pauli, M. and Bazerque, P. 1990. An antibiotic assay by the agar-well diffusion method. Acta Biologiae. Medecine Experimentalis 15:113-115.

Santoyo, S., Lioria, R., Jaime, L., Ibanez,E., Senorans, F.J. and Reglero, G. 2006.

Supercritical fluid extraction of antioxidant and Antimicrobial compounds from *Laurus nobilis* L. Chemical and functional characterization. European Food Res. Technol, 222: 565– 571.

Sarika, S.S., Sanket R.J. and Aarti, G.J. 2008. Methanolic Extract of *Cuminum cyminum* inhibits Ovariectomy-Induced Bone Loss in Rats. Experimental Biology and Medicine, 233(11): 1403-1410.

Shrinivas, H., Bagul, M.S., Padh, H. and Rajani, M. 2004. A Rapid Densitometry Method for the Quantification of Luleolin in Medicinal Plants Using HPTLC. *Chromatographia*, 60(1-2): 131-134.

Tonk, S., Bartarya, R., Maharaj, K.K., Bhatnagar, V. P. and Srivastava, S.S. 2006. Effective method for extraction of larvicidal component from leaves of *Azadirachta indica* and *Artemisia annua* Linn. Journal of Environmental Biology 27 (1): 103-105.