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Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE FRUITS OF *CUCUMIS TRIGONUS* ROXB.

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ABSTRACT
The antibacterial and antifungal activities of the successive extracts (petroleum ether (40-60ºC), benzene, chloroform, ethanol and water) of the fruit of *Cucumis trigonus* Roxb. (Fam. *Cucurbitaceae*) have been carried out against three gram positive bacteria, *Bacillus cereus, Baccillus subtilis, Streptococcus faecalis*, three gram negative bacteria, *Pseudomonas aeruginosa, Klebsiella aerogenos, Proteaus vulgaris* and two fungi, *Candida albicans, Asparagillus flavans* by using disk diffusion method. Zones of inhibition of the extracts have been compared with that of the standard antibiotics. The petroleum ether (40-60ºC) and chloroform extracts showed no activity while the ethanolic extract showed more activity than the benzene and aqueous extracts. Minimum inhibitory concentration (MIC) for the ethanolic extract of the fruit of *Cucumis trigonus* has also been determined. The results indicate that *Cucumis trigonus* is a potential antiseptic for the prevention and treatment of microbial infections.
Keywords: *Cucumis trigonus,* Disk diffusion method, Antimicrobial, Antifungal Minimum inhibitory concentration.

INTRODUCTION

Cucumis trigonus Roxb. (Fam. *Cucurbitaceae*) commonly known as "Thummittikai" in Tamil, "Vishala" in Sanskrit and "Bitter gourd" in English is reported to possess a number of medicinal values[1]. In Indian traditional systems of medicine the fruit pulp of the plant is used as expectorant, liver tonic, stomachic and purgative. The fruit pulp is useful in leprosy, jaundice, diabetes, bronchitis and amentia[2]. Fruit pounded and boiled with cow's milk and applied to the head is supposed to prevent insanity, strengthen the memory and remove vertigo. The drug is also used in snake bite.

The present work deals with the effect of various extracts of *C. trigonus* on pathogenic strains of gram-positive bacteria (*Streptococcus faecalis, Baccillus subtilis* and *Baccillus cereus*) gram negative bacteria (*Pseudomonas aeruginosa, Klebsiella aerogenos and Proteaus vulgaris*) and fungi (*Candida albicans, Asparagillus flavans)* by using zone of inhibition assays[3]. The corresponding solvents are used as solvent control. The antibiotics Streptomycin and Clotrimazole are used as standard for bacteria and fungi respectively.

MATERIAL AND METHODS

Plant materials

The plant was collected in the month of March from Alangulam, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Center, West Thambaram, Chennai- 600 045, Tamil Nadu.

A voucher specimen (MSU/PHAR/HER–140) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012.

Extraction of plant material

The collected fruits were cut into pieces, shade-dried at room temperature and powdered. The dried fruit powder (500 gm) was successively extracted using petroleum ether (40°-60°C), benzene, chloroform, ethanol and water by using Soxhlet apparatus. The last trace of solvent was removed under reduced pressure distillation and then vacuum dried. The dried crude extracts were used for the study.

Micro-organisms used

Bacterial strains used for testing included *Streptococcus faecalis* (MTCC 459), *Baccillus subtilis* (MTCC 619), *Baccillus cereus* (MTCC 430), *Proteaus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenos* (MTCC 530). The fungi used were *Candida albicans* (MTCC 183), *Asparagillus flavans* (MTCC 1973). These were obtained from Central Research Institute, Khashuli, Chandigarh, Gujarat, India. The stock culture was maintained on Mueller Hinton agar medium (Himedia Chemicals) at 37°C.

Preparation of the test organisms

The bacterial and fungal cultures were incubated for 24 h at 37°C in nutrient agar slants (Himedia, Mumbai, India) respectively. Before streaking, each culture was diluted (1:10) with fresh sterile nutrients broth. Plates were prepared by pouring 20 ml of freshly prepared No.1 medium (Himedia, Mumbai, and India) into 20 mm x 100 mm Petri plates. Inoculums (5 ml) was poured directly over the surface of prepared plates to uniform depth of 4 mm and then allowed to solidify at room temperature.

Antimicrobial Assay

The antimicrobial activity was determined by the Paper disc diffusion method[4]. A suspension of the organism was added to sterile nutrient agar medium at 45°C. The mixture was transferred to sterile Petri plates and allowed to solidify. Sterile disc, 5 mm in diameter (made from whatmann filter paper previously sterilized in UV-lamp) was dipped in test drug solution. 1000 µg of each extract was prepared by dissolving 10 mg of each extract separately in 10 ml of the respective solvents. Then the sterile disc containing each test drug solution of the plant extract (200 µl) was placed over the seeded agar plates in such a way that there is no overlapping of zone of inhibition. Standards and a blank were placed on the surface of agar plate[5]. The antibiotics, Streptomycin (10 µg) and Clotrimazole (10 µg) were used as standards for bacteria and for fungi respectively. The plates were kept at room temperature for two hours to allow diffusion of the test drug into the agar. They were incubated for 24 and 48 h at 37° C for the bacterial and fungal strains respectively. After the incubation period was over, the plates were observed for

Zone of Inhibition (ZI) measured in millimeters (mm). From the results the Activity Index (AI) and Proportion Index (PI) were calculated using the following formulae:-

$$\text{Activity Index(A1)} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of standard}}$$

$$\text{Proportion Index} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the ethanolic extract of *Cucumis trigonus* was determined in µg/m[6, 7]. The samples of the extract were prepared at five different concentrations, 1000 µg/ml, 800 µg/ml, 600 µg/ml, 400 µg/ml and 300 µg/ml. 90% Ethanol was used as the solvent control.

RESULTS AND DISCUSSION

In vitro preliminary screening of the antimicrobial activity of the various extracts of the fruits of *Cucumis trigonus* Roxb. were studied against the micro-organisms using the filter paper disc diffusion method. The antimicrobial effects of fruit extracts against the different strains are presented in Table 1. From Table 1, it is clear that both the ethanol and water extracts inhibited the growth of all the tested strains of bacteria and fungi. The benzene extract was effective only in the gram negative bacteria and fungi. The petroleum ether and the chloroform extracts were resistant to all the tested strains of bacteria and fungi. The ethanol extract showed the maximum activity against all the tested strains used in the present study followed by water extract. The difference in the activity may be due to the different secondary metabolites present in the ethanol and the water extracts.

The ethanol extract showed the highest activity against both the fungi, *Candida albicans* (16 mm) and *Asparagillus flavans* (16 mm) followed by the gram negative bacteria, *Klebsiella aerogenos* (15 mm), *Pseudomonas aeruginosa* (15 mm). Similar activity was noticed in the cases of *Proteaus vulgaris* (14 mm), and *Streptococcus faecalis* (14 mm). The water extract showed the same activity in the cases of *Klebsiella aerogenos* (11 mm), *Pseudomonas aeruginosa* (11 mm). The lowest activity is noticed in the cases of *Baccillus subtilis* (6 mm) and *Proteaus vulgaris* (6 mm).

The proportion index of the antimicrobial activity of the various extracts of the fruits of *Cucumis trigonus* is presented in Fig. 1.

Minimum inhibitory concentration of the ethanolic extract of *Cucumis trigonus* is presented in Table 2. From the results, it is clear that the MIC value for both the gram-positive and gram-negative bacteria was found to be 400 µg/ml. The ethanol extract of the fruits of *Cucumis trigonus* showed better activity in the case of fungi than the bacteria.

CONCLUSION

The results obtained in the present investigation demonstrated that the fruits of *Cucumis trigonus* display *in vitro* antimicrobial activity. The traditional uses of the fruits for infections and other therapeutic claims have been confirmed by the antimicrobial activity. Hence the plant drug can be used as a safe alternative to synthetic antibiotics.

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Table 1. Antimicrobical Activity of the various extracts of the fruits of *Cucumis trigonus* Roxb.

SI.No.	Name of the Organisms	Zone of Inhibition of the extracts (mm) and Activity Index										
		Petroleum ether (40°-60°C)		Benzene		Chloroform		Ethanol		Water		Standards
		ZI	AI	ZI	AI	ZI	AI	ZI	AI	ZI	AI	
1.	*B. cereus*	-	0	-	0	-	0	13	0.72	7	0.39	18[a]
2.	*B. subtilis*	-	0	-	0	-	0	11	0.61	6	0.33	18[a]
3.	*S. faecalis*	-	0	-	0	-	0	14	0.77	12	0.67	18[a]
4.	*K. aerogenos*	-	0	5	0.26	-	0	15	0.79	11	0.58	19[a]
5.	*P.aeruginosa*	-	0	5	0.26	-	0	15	0.79	11	0.58	19[a]
6.	*P. vulgaris*	-	0	6	0.32	-	0	14	0.74	6	0.32	19[a]
7.	*C. albicans*	-	0	6	0.33	-	0	16	0.89	11	0.61	18[b]
8.	*A. flavans*	-	0	6	0.32	-	0	16	0.84	10	0.52	19[b]

a – Streptomycin; b – Clotrimazole; ZI-Zone of Inhibition; AI-Active Index; - No inhibitory effect

Table 2: MIC of the ethanolic extract of the fruits of *Cucumis trigonus* Roxb.

SI.No.	Name of the organisms	Zone of Inhibition (mm)				
		300 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
1.	B. *cereus*	-	06	08	09	13
2.	B. *subtilis*	-	05	07	10	11
3.	S. *faecalis*	-	06	09	11	14
4.	K. *aerogenos*	-	07	12	14	15
5.	P.*aeruginosa*	-	07	12	14	15
6.	P. *vulgaris*	-	06	12	13	14
7.	C. *albicans*	07	09	13	15	16
8.	A. *flavans*	06	08	12	14	16

- No inhibitory effect

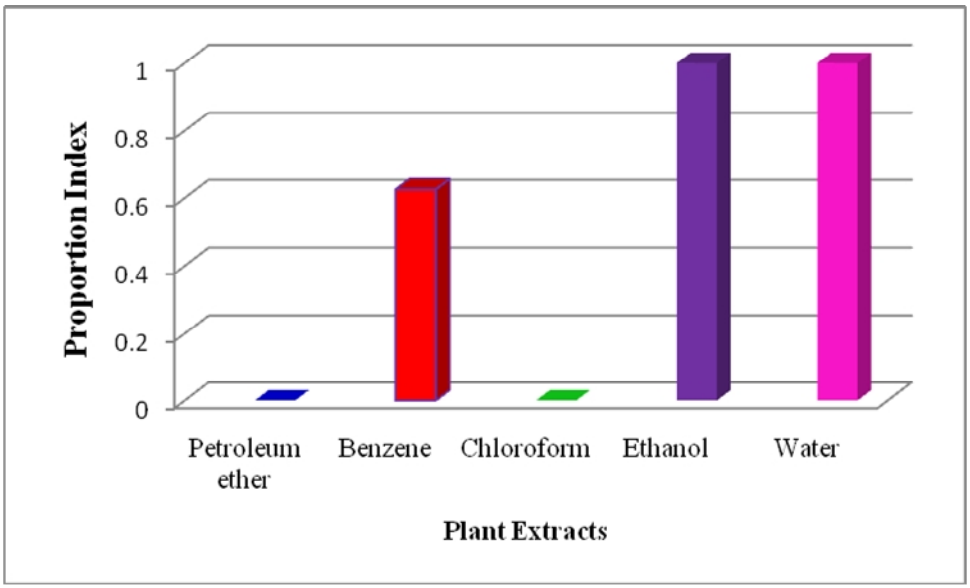

Fig 1: Proportion Index of antimicrobial activity of the various extracts of the fruits of *Cucumis trigouns* Roxb.

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