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Research Article

SCREENING OF VARIOUS EXTRACTS OF THE FRUITS OF CUCUMIS SATIVUS LINN. FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

To evaluate the antimicrobial activity of the various extracts of the fruits of Cucumis sativus Linn. Materials and methods: Antimicrobial activities of petroleum ether (40-60ºC), benzene, chloroform, ethanol and water extracts of the fruits of Cucumis sativus (L) have been investigated against both bacteria and fungi using the disc diffusion method. Results: The results of investigation showed that ethanol, and aqueous extract showed inhibitory effect on the growth of all the isolates. There was no inhibitory effect of the petroleum ether, benzene, and chloroform extract against the test organisms. Ethanolic extract of the fruits of Cucumis sativus had a minimum inhibitory concentration (MIC) of 200 µg/ml against three gram positive bacteria, viz., Baccillus cereus, Baccillus subtilis, Streptococcus faecalis, three gram negative bacteria, viz., Pseudomonas aeruginosa, Klebsiella aerogenos, Proteaus vulgaris and the two fungi, Candida albicans, Asparagillus flavans. Streptomycin, and Clotrimazole were used as standards for bacteria and fungi respectively. Conclusion: The results shows that the ethanolic extract of Cucumis sativus can be a potential source of natural antimicrobial agent.

Keywords: Antimicrobial, Cucumis sativus, Disk diffusion method, Zone of inhibition, Minimum Inhibitory Concentration (MIC).

INTRODUCTION

Medicinal plants have been used as sources of medicine in virtually all cultures (Baquar, 1995). In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different Indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Kubra Karakoca et al., 2013). The cucumber is the edible fruit of the cucumber plant Cucumis sativus, which belongs to the gourd family Cucurbitaceae. The cucumber plant is an annual climber which grows to a height of 15-30 cm and has large leaves that form a canopy over the fruit. Cucumbers contain usually more than 90 % water. The plant Cucumis sativus is commonly known as “Mullu vellari” in Tamil, “Sakusa” in Sanskrit and “Cucumber” in English. The plant is a creeping vine which bears cylindrical edible fruit. Fruits are located at different distances from the root system. The juice is used in many beauty products (Katsambas & Lotti, 2003). Cucumis sativus fruit is shown to possess various activities such as cytotoxic activity and antifungal activity (Jony Mallik et al., 2012), antacid and carminative activity (Swapnil Sharma et al., 2012), hepatoprotective activity (Gopalakrishnan et al., 2013), hypoglycemic and hypolipidemic activity (Sharmin et al., 2013), wound healing activity (Patil et al., 2011). In the present work, the antimicrobial activity of the various

extracts of the fruits of Cucumis sativus has been reported for the first time.

MATERIAL AND METHODS

Plant materials

The plant Cucumis sativus was collected in the month of July from Alangulam, Tirunelveli-627 012, 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–141) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012, Tamil Nadu.

Extraction of plant material

The 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.

Microbial strains

Bacterial strains used for testing included Streptococcus faecalis (MTCC 459), Baccillus subtilis (MTCC 619), Baccillus cereus (MTCC 430), Proteaus vulgaris (MTCC 771), Pseudomonas aeruginosa (MTCC 741), Klebsiella aerogenos (MTCC 530). The fungi used were Candida albicans (MTCC 183), Asparagillus flavans (MTCC1973). 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.

Disc-diffusion assay: The antimicrobial activity of the various extracts of the fruits of Cucumis sativus was determined by the Paper disc diffusion method (Ayandele et al., 2007). 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 (Paniker and Anantharaman, 2005). 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 h and 48 h at 37oC 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 (P.I) were calculated using the following formulae:-

$$\text{Activity index (AI)} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of standard}}$$

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

Determination of the minimal inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the ethanolic extract of the fruits of Cucumis sativus were determined in µg/ml (Okeke et al., 2001). The samples of the extract were prepared at five different concentrations, 1000 µg, 800 µg, 600 µg, 400 µg and 200 µg/ml. The solvent, 90 % Ethanol was used as a solvent control.

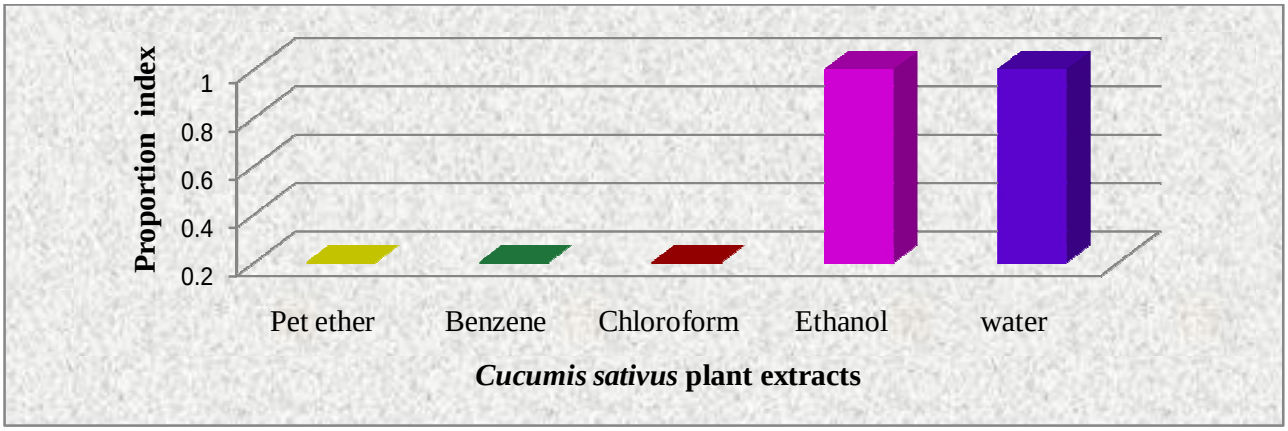

Fig 1. Proportion Index of antimicrobial activity of the various extracts of the fruits of *Cucumis sativus* Linn.

Table 1. Antimicrobical Activity of the various extracts of the fruits of *Cucumis sativus* Linn.

Sl.No.	Name of the Organisms	Zone of Inhibition of the extracts (mm) and Activity Index										
		Petroleum ether (40°-60°C)		Benzene		Chloroform		Ethanol		Water		Standard
		ZI	AI	ZI	AI	ZI	AI	ZI	AI	ZI	AI	
1.	*B. cereus*	-	0	-	0	-	0	10	0.67	11	0.73	15[a]
2.	*B. subtilis*	-	0	-	0	-	0	11	0.73	09	0.60	15[a]
3.	*S. faecalis*	-	0	-	0	-	0	11	0.73	08	0.53	15[a]
4.	*K. aerogenos*	-	0	-	0	-	0	12	0.71	07	0.41	17[a]
5.	*P.aeruginosa*	-	0	-	0	-	0	13	0.76	08	0.47	17[a]
6.	*P. vulgaris*	-	0	-	0	-	0	14	0.82	10	0.59	17[a]
7.	*C. albicans*	-	0	-	0	-	0	12	0.67	08	0.44	18[b]
8.	*A. flavans*	-	0	-	0	-	0	13	0.72	09	0.50	18[b]

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

Table 2. MIC determination (200 µg/ml to 1000 µg/ml) for samples of the ethanolic extract of the fruits of *Cucumis sativus* Linn.

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

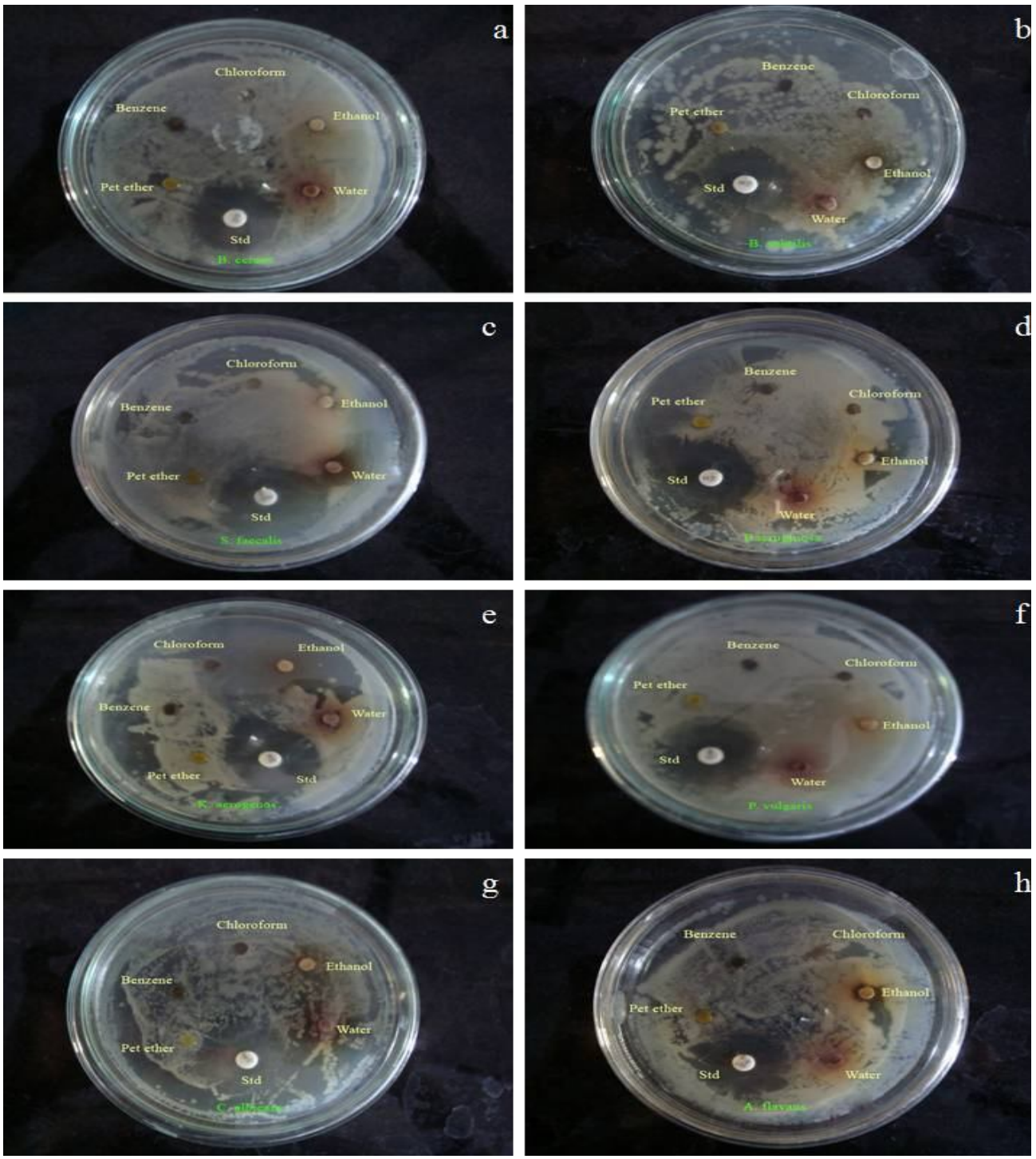

Fig 2. Effect of various extracts of the fruits of *Cucumis sativus* Linn. against various microorganisms.

a). *B. cereus*

b). *B. subtilis*

c). *S. faecalis*

d). *P.aeruginosa*

e). *K. aerogenos*

f). *P. vulgaris*

g). *C. albicans*

h). *A. Flavans*

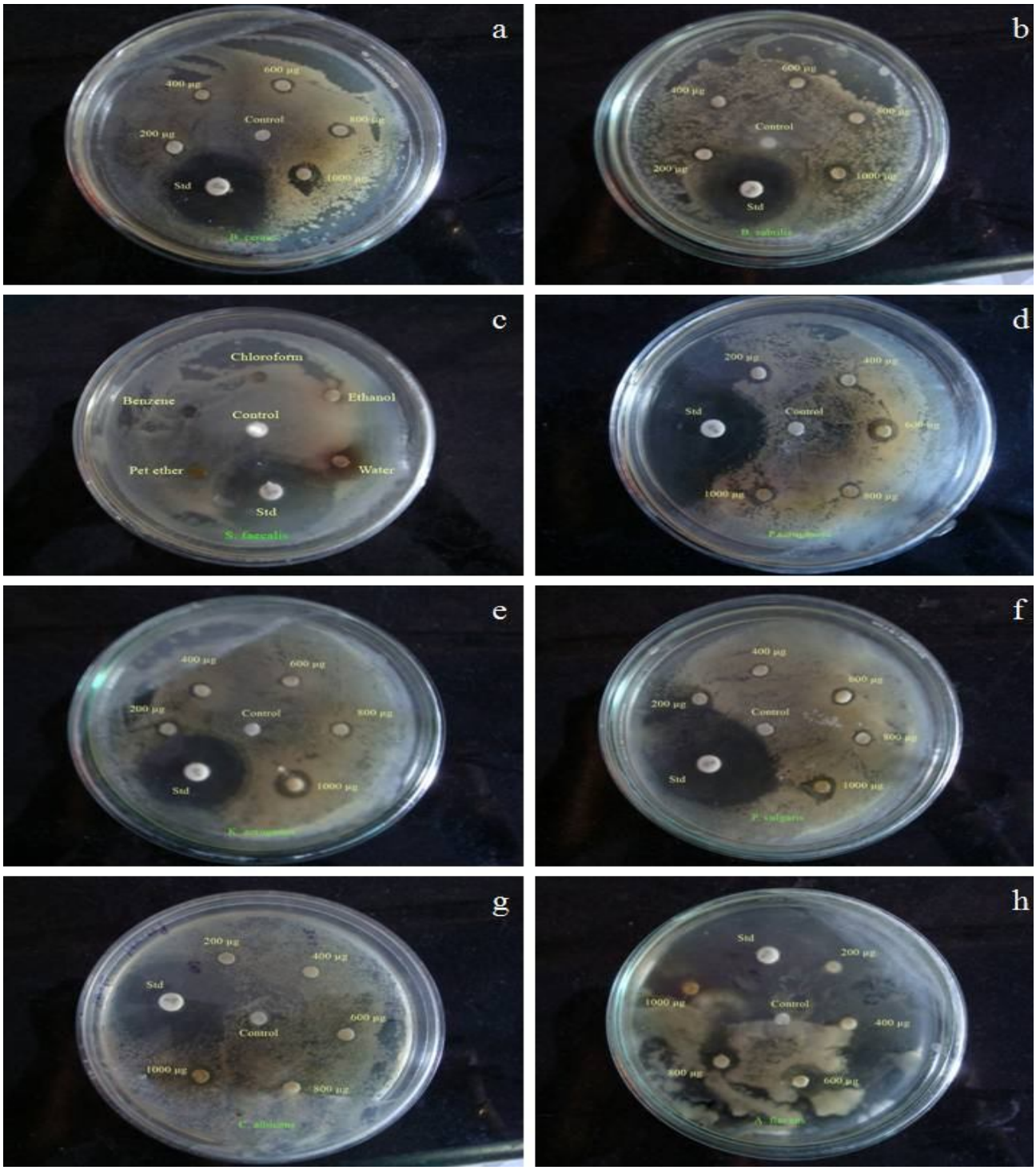

Fig 3. MIC of ethanolic extract of the fruits of Cucumis sativus Linn. against various microorganisms.

a). B. cereus

b). B. subtilis

c). S. faecalis

d). P.aeruginosa

e). K. aerogenos

f). P. vulgaris

g). C. albicans

h). A. Flavans

RESULTS AND DISCUSSION

The antimicrobial activity showing the zones of inhibition in millimeters for gram-positive, gram-negative bacteria and fungi are presented in Table 1. The data obtained from the disk diffusion method some of the extracts exhibited no antimicrobial activity against both the test bacteria and fungi. The ethanol extract and water extract of the fruits of Cucumis sativus possess significant inhibitory activities against all the gram-positive, gram-negative bacteria and fungi.

There was no significant antibacterial activity of the pet ether, benzene, and chloroform extract against the test organisms. The highest and weakest inhibitory activity was determined for ethanol extract against the gram negative bacteria P. Vulgaris (14 mm) and the gram positive bacteria B.cereus (10 mm) respectively. On the other hand, for aqueous extract the highest inhibitory activity was determined against gram positive bacteria B.cereus (10 mm) and the weakest inhibitory activity was determined against gram negative bacteria K. aerogenos (7 mm). The ethanol extract showed higher inhibitory activity than the aqueous extract. The minimum inhibitory concentration of ethanolic extract of the fruits of Cucumis sativus ranged from 200 µg/ml to 1000 µg/ml (Table 2). The results showed that increase in concentration of the extract leads to increase in zone of inhibition.

The proportion index of the antimicrobial activity of the various extracts of the fruits of Cucumis sativus is presented in Fig 1. Effect of various extracts and minimum inhibitory concentration of ethanolic extract of the fruits of Cucumis sativus against various microorganism are presented in Fig 2 and Fig 3. The antimicrobial activity of the ethanolic extract of the fruits of Cucumis sativus may be due to the presence of 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 5-(Hydroxylmethyl) -2-furancarboxyaldehyde, 4-Hydroxy -3-methyl-2-butenyl-acetate, 2-(2-Methylcyclohexylidene)-hydrazinecarboxamide, 1,2-Benzene dicarboxylic acid-diisooctyl ester which were found by GC-MS (Gopalakrishnan et al., 2013).

CONCLUSION

The results obtained in the present investigation demonstrated that the fruits of Cucumis sativus display in vitro antimicrobial activity. The traditional use of the fruits is applied to the skin as a cleansing cosmetic to soften and whiten it. The seeds of these plants are diuretic and anthelmintic effect. Hence the plant extracts possess compounds with antibacterial properties that can be used as very good antimicrobial agents.

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