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Evaluation of antimicrobial properties and phytochemical constituents of *Capsicum frutescens* L.

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ABSTRACT

In this study, the crude extract of leaf and fruit material of *Capsicum frutescens* L. were extracted with various solvents and subjected to antifungal activity by poison food technique against the five pathogenic fungi, namely *Aspergillus flavus, Fusarium oxysporium, Curvularia lunata, Alternaria alternata and Chaetomium globosum.* The activity of fruit extract was high when compare to leaf extract. The antibacterial activity were done by Agar well diffusion method against human pathogenic fungi i.e. *Pseudomonas aeruginosa and Salmonalla typhimurium* and plant pathogenic fungus *Xanthomonas comprestris*, fruit extract shows high inhibitory zone against human pathogens than the leaf. The activity of leaf and fruit extract was least on plant pathogen.

KEYWORDS

Antibacterial;
C.frutescens;
Fusarium;
Phytochemicals.

INTRODUCTION

Vegetables are high value crops and often provide excellent income generating opportunities to small farmers[1]. *Capsicum frutescens* L. and *Capsicum annum* L. commonly known as Chilli is among the world's most popular vegetable of solanaceous family after potato and tomato and is being used mainly as spices and condiments[2]. In India, it is a significant cash crop occupying 15% of the total area under vegetable cultivation. The genus capsicum consists of approximately 22 wild species and 5 domesticated species, *C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens*. Capsicum is a perennial shrub, Chilli fruit are considered as vegetables with high economic value. Chilli types usually classified by fruit characteristic that is pungency, color, size, shape, flavour and their use. Despite their vast trait differences most Chilli cultivars commercially cultivated in the world belong to the species *C. annuum.*

The level of pungency of the capsicum species depends upon the concentration of capsaicinoids, primarily of capsaicin, in the fruit. The chemical composition of capsicum is a fixed oil, pungent principles, volatile oil and carotenoids, mostly capsanthin pigments. An oleoresin is obtained by solvent extraction. *C. frutescens* L. is much more pungent then *C. annuum* L. They are used as rubefacient, antisparmodic, carminative, antioxidant, antimicrobial, stypt, etc., because of its valuable and medicinal importance it is selected for study[3].

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MATERIALS AND METHOD

Collection and extraction

The Fruit and leaf samples were collected from the village Harsikatta near Siddhapur, Karnataka. The plants were authenticated in Dept. of P.G. Studies and Research in Applied Botany, Jnana Sahyadri, Kuvempu University, Shankaraghatta and voucher specimens (KU/AB/SH/331) were deposited in the department for future reference. The plant materials were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for extraction. The powdered plant material was extracted with solvents namely petroleum ether, chloroform and methanol. A known amount of powdered material (500gm) was subjected to soxhlet extraction and exhaustively extracted with respective solvents for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccators. To assess the efficiency of the active principles, antimicrobial activity was carried out[4-6].

Antifungal assay

Antifungal activity by poison food technique[7,8]. The test fungi was allowed to grow in potato dextrose agar media plates poisoned with extracts. The test fungi (7 day old culture) were inoculated by point inoculation method. The effect of extraction on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with control (plates not poisoned with extract). The experiment was done in triplicates and average reading was recorded. The fungal species like *Aspergillus flavus[*MTCC-3793], *Fusarium oxysporium[*MTCC-6062], *Curvularia lunata[*MTCC-4627], *Alternaria alternata[*MTCC-3793] and *Chaetomium globosum*[MTCC-4179] were tested for their susceptibility to the solvent extracts. All these cultures were obtained from MTCC collection IMTECH (Institute of Microbial Technology) Chandigarh, India. The cultures were maintained at 4°C and subculture frequently in respective media.

Preliminary screening of solvent extracts for antibacterial activity

The sterile nutrient agar plates were taken, the 24hrs old culture of test bacteria from broth culture was inoculated on the solidified agar by swabbing uniformly and wells of 6 mm were bored. DMSO was used as control. The extract of concentration 25µg/ml, 50µg/ml and 100µg/ml were prepared in DMSO. Tetracycline (1gm/ml) was used as standard drug. The control, standard and extract were allowed to stand for 20 min for diffusion. Then the plates were kept in incubator for 24hrs at 37C in an upright position. After incubation, the zone of inhibition formed around the wells was measured. The experiment was carried in triplicates to arrive concordant values[9] methods. The microbes like *Xanthomonas comprestris[*MTCC-2286] a plant pathogen, *Psudomonas aeruginosa[*MTCC-424] and *Salmonalla typhimurium[*MTCC-3214] Gram negative pathogenic bacteria were used. The bacteria were procured from Institute of Microbial Technology, Chandigarh.

Determination of minimum inhibitory concentration (MIC)

The MIC of the solvent extracts against test bacteria was tested by Agar well diffusion method[9]. The 24 hours old standardized Muller-Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Then, aseptically wells of 6 mm diameter were bored in the inoculated plates with the help of gel puncher and various dilutions of the extract (25, 50 and 100µg/ml of DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. The least dilution among four was considered as the MIC. The experiment was carried in triplicates to get average reading.

RESULTS AND DISCUSSION

The presence of various secondary metabolites in the methanol extract of plants selected for study is given in (TABLE 1). The phytoconstituents namely alkaloids, steroids, saponins, and glycosides are found in the *C. frutescens,* but tannins, terpenoids, triterpenoids and flavinoids are absent.

The extract of fruits and leaves were subjected for antifungal and antibacterial activities. Five fungal member's *A. flavus, F. oxysporium, C. lunata, A. alternata, C. globosum* were used as test organisms.

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TABLE 1 : Phytochemical constituents in the different solvent extracts of *C. frutescens*

Extract	Saponins	Tannins	Alkaloids	Terpenoids	Triterpenoids	Glycosides	Steroids	Flavinoids	Carbohydrates	Protein
Methanol. Fruit	-	-	+	-	-	+	+	-	+	+
Ethanol Fruit	-	-	+	-	-	+	+	-	+	+
Pet.Ether. Fruit	-	-	+	-	-	+	+	-	+	+
Chloroform. Fruit	-	-	+	-	-	+	+	-	+	+
Methanol. Leaf	+	-	+	-	-	+	+	-	+	+
Ethanol. Leaf	+	-	+	-	-	+	+	-	+	+
Pet.Ether. Leaf	+	-	+	-	-	+	+	-	+	+
Chloroform. Leaf	+	-	+	-	-	+	+	-	+	+

TABLE 2 : Preliminary screening for antimicrobial activity of solvent extracts of *C. frutescens*

Extract	Plant pathogen	Human Pathogen			Pathogenic Fungi			
	X. compestris	*S. typhi*	*P. aureginosa*	*A. flavus*	*F.oxysporium*	*C.lunata*	*A. alternata*	*C. globosum*
Control	-	-	-	-	-	-	-	-
Stantard	+	+	+	+	+	+	+	+
Methanol Fruit	+	+	+	-	-	-	-	-
Ethanol Fruit	+	+	+	-	-	-	-	-
Petroleum Ether Fruit	+	+	+	-	-	-	-	-
Chloroform Fruit	+	+	+	-	-	-	-	-
Methanol Leaf	+	+	+	+	+	+	+	+
Ethanol Leaf	+	+	+	+	+	+	+	+
Petroleum Ether Leaf	-	+	+	+	+	+	+	+
Chloroform Leaf	-	+	-	+	+	+	+	+

The Methanol extract was shown maximum inhibitory effect on *C. globosum* and minimum on *A. flavus*. Ethanol extract of leaf inhibited maximum growth of *C. lunata*. The petroleum ether extract of leaf inhibited maximum growth of *A. alternata* and *C. globosum* and minimum growth of *F. oxysporium*. The chloroform extract of leaf inhibited maximum growth of *C. lunata* and *C. globosum* and minimum growth of *A. flavus* (TABLE 2), at the concentration of 100mg/ml of fruit extract, fruit extract shows high inhibition (no growth) and leaf shows least inhibition for all the experimental fungal species.

Among the entire extracts inhibition zone was found in increasing order with increase in concentration. The activity of fruit extracts showed comparatively higher inhibition zone than leaf extracts. The inhibition activity was high in human pathogen than plant pathogen. However all the solvent extract of fruit inhibited growth of *C. lunata, A. alternata* and *C. globosum*. However extracts of methanol ethanol and chloroform inhibited the growth of *A. flavus* and *F. oxysporium* and petroleum ether extract of fruit partially inhibited the growth of *A. flavus* and *F. oxysporium*. Therefore the study reveals that the fruit

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TABLE 3 : Antifungal activity (In terms of zone of inhibition in mm) of various extracts of *C. frutescens*

Extract	*A. flavus*	*F.oxysporium*	*C.lunata*	*A. alternata*	*C. globosum*
Standard	15	38	40	34	25
Methanol Fruit	-	-	-	-	-
Ethanol Fruit	-	-	-	-	-
Pet. Ether Fruit	2	2	-	-	-
Chloroform Fruit	-	-	-	-	-
Methanol Leaf	8	16	8	3	2
Ethanol Leaf	4	6	8	8	2
Pet. Ether Leaf	3	17	8	2	2
Chloroform Leaf	8	13	2	3	4

extracts of different solvents can be used as antifungal agents. As it is stated that all the solvents extracts of fruit inhibited growth of all the fungus, where as the leaf extract was maximum on *C. globosum* and minimum on *F. oxysporium*.

The result of antibacterial activity of solvent extracts is shown in TABLE 3. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it is reported as positive and absence of zone as negative[10]. It was found that all the solvent extracts of the plants, except petroleum and chloroform leaf extract of *C. frutescens* were found to inhibit growth of test bacteria. Standard antibiotic Tetracycline (1mg/ml) has shown inhibition of all tested bacteria. The control solvent DMSO did not reveal inhibition of any of the tested bacteria.

The antibacterial activity of C. frutescens has been evaluated not only on plant pathogens (*X. compestris*), but also on human pathogens (*S. typhi* and *P. aureginosa)*. The control antibiotic (Tetracycline) on the fruit and leaves extract of *C. frutescens* were used in the concentration of 25, 50 and 100 mg/ml both on plant pathogen and human pathogens. The petroleum and chloroform extract of leaf did not show any inhibitory effect on plant pathogen where as ethanol extract of leaf and chloroform extract of leaf showed inhibitory effect on plant pathogen *X. compestris* and *P. aureginosa*, a human pathogen, at higher concentrations. However, the fruit extract of all the solvent extracts inhibited the growth of both plant pathogen and human pathogens. It is also true that the concentration of extracts of both fruits and leaves and inhibitory effects are directly proportional (TABLE 4).

TABLE 4 : Antibacterial activity (Zone of inhibition in mm) of various extracts of *C. frutescens*

Plant pathogen				Human Pathogen					
Extract	*X. compestris*			*S. typhi*			*P. aureginosa*		
Amount In mg/ml	25	50	100	25	50	100	25	50	100
Control	-	-	-	-	-	-	-	-	-
Tetracycline	8	10	15	8	8	15	8	10	15
Methanol Fruit	4	5	6	4	7	7	3	3	6
Ethanol Fruit	3	4	6	3	6	9	2	3	5
Pet. Ether Fruit	2	4	5	3	5	8	2	4	5
Chloroform Fruit	2	3	5	3	5	8	3	4	5
Methanol Leaf	1	1	3	2	4	6	2	3	4
Ethanol Leaf	-	-	2	2	3	4	2	3	4
Pet. Ether Leaf	-	-	-	2	3	5	1	2	4
Chloroform Leaf	-	-	-	2	3	5	-	2	3

Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents[11]. In our study, the extracts have shown promising results in terms of inhibition of bacteria tested. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens.

CONCLUSION

The plant materials selected for this study have shown a good activity against the tested bacteria and fungi. The extracts can be used to treat infections caused by these bacteria. The presence of various phytoconstituents in the extracts highlights the antibacterial efficacy of the plants. The extracts can be preferred in the treatment of several types of infections such as nosocomial infections, enteric infections, urinary tract infections, food poisoning, wound infections etc. Further studies are to be carried out on animal models.

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