



PHYTOCHEMICAL EXPLORATION OF CAPSICUM FRUTESCENS L. AND CAPSICUM FASTIGIATUM L. AND ITS ANTIMICROBIAL POTENTIAL IN CHOSEN PATHOGENIC ORGANISMS

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Abstract : The Indian medicinal plants, like *Capsicum frutescens* L. and *Capsicum fastigiatum* L. were examined using agar disc diffusion method against E.coli and Enterobacter sp. Plant leaves and stalks were extracted using different solvents such as, butanol, ethanol and water. Phytochemical screening of these plants was performed for constituents like alkaloids, flavonoids, tannins, saponins, sugar, reducing sugar, aminoacids, steroids and phenolic compounds. Among the two plants the *Capsicum frutescens* have more photochemical constituents compared to *Capsicum fastigiatum* .Among the different extracts, ethanol extract showed more antibacterial activity and moderate activity recorded with butanol and water extracts. *Capsicum frutescens* showed maximum antibacterial activity against two tested bacteria than the other plant. Two bacteria were more susceptible to ethanol extract than the other organic extracts. Therefore, it may be recommended for the preparation of plant based drugs against human bacterial pathogens. In future these plants can be subjected to isolation of the major constituent's antimicrobials, antidiabetic, anticancer and to further pharmacological evaluation.

IndexTerms *Capsicum frutescens* L. and *Capsicum fastigiatum* L. phytochemical constituents, antimicrobial activity, E.coli and Enterobacter sp.

1. INTRODUCTION

Phytochemical are nonnutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemical can protect humans against diseases. There are many phytochemical in fruits and herbs and each works differently. (Jayashree and Maneemegalai, 2008). According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenol compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyar *et al.*, 2006).

Environment is the interaction between man and the nature. Human beings are surrounded by people, animals, plants and physical objects which are parts of our environment. Many medicinal plants which are used to manufacture of new antibiotics in pharmaceutical industry. These are resistance to microorganisms. Nature has been of material agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg and Newman, 2001 and Alam *et al.*, 2009). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics (Nascimento *et al.*, 2000).

Capsicum frutescens L. (Caesalpinaceae) is an annual foetid herb, with a height of 30 to 90 cm, it is mainly found in the states of Uttar Pradesh and Madhya Pradesh, in India. It has pinnate leaves, which are

about 10 cm long. *Capsicum fastigiatum* L. (Solanaceae) Capsicum is a stimulant. Rapidly increases capillary circulation to a part when applied. Taken internally by its stimulating properties, it promotes its own absorption and thus produces its effect on the nerve centers. It increases the tone of the entire system; increases circulation and produces a feeling of warmth all over the body. On account of its local and general effects it is indicated in atonic conditions, relaxed muscular fibers, and a general deficiency of functional force. We think of it in dipsomania, delirium tremens, malaria, congestive chills and atonic dyspepsia. In malignant intermittent fever combined with quinine it is one of our very best remedies. It is best given in cream or milk as it is less irritating to the mucous membrane in this form. Antimicrobial activity of various extracts of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. against human bacterial pathogens were reported by several workers (Sharma *et al.*, 2006; Saravanan *et al.*, 2008; Manjula *et al.*, 2009, Prasad *et al.*, 2009; Khan *et al.*, 2010 and Saurabh *et al.*, 2011). In the present investigation was *Capsicum frutescens* L. and *Capsicum fastigiatum* L. extracted with a low polar to high polar organic solvents and against human bacterial pathogens.

The search for antimicrobials of plant origin has been mainly stimulated by the fact that some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum. Now-a-days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Lakshmi Naidu *et al.*, 2006). To date, resistance in bacteria is most prevalent. For example, methicillin resistant *Staphylococcus aureus* (MRSA) has become a huge problem worldwide to treat nosocomial infections since 1990s (Lee *et al.*, 2007). Therefore, in the present investigation the Phytochemical and its antimicrobial activity of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. was assessed against human bacterial pathogens.

2. MATERIALS AND METHODS

Phytochemical activity

The leaves of *Capsicum frutescens* L. and stalk of *Capsicum fastigiatum* L. extracted with various solvents were used to screen the following phytochemicals like sugar, reducing sugar, tannins, alkaloids, saponins, amino acids, steroids, flavonoids and phenolic compounds

Sugar

The test extracts were treated with minimum quantity of anthrone and a few drops of concentrated sulphuric acid and then heated. Change color from green to purple showed the presence of sugar.

Reducing sugar

The test extracts were treated with 2ml of Fehling's reagent and 3ml of water. The test content was boiled and the development of red orange color indicated the presence of reducing sugar.

Tannins

The test extracts were treated with water and basic lead acetate. The presence of tannins in the test solution was noticed by the development of white ppt.

Saponins

The test extracts were treated with water shakes well. The test solution was changed into foamy ether, if the test sample contains saponins.

Amino acids

After treating the extract with ninhydrin in alcohol. The violet colour formed confirmed the presence of amino acids.

Flavonoids

A bit of magnesium and then one or two drops of concentrated hydrochloric acid were added. To the plant extract was heated and the development of red or orange color indicated the presence of flavonoids.

Phenolic compounds

The test extract in alcohol was treated with a drop of neutral ferric chloride. Change of intense color in the test content, shows positive result for phenolic compounds.

Alkaloids

Aqueous layer was formed when the test extracts were treated with 2N hydrochloric acid. It was decanted and to which one (or) two drops of Mayer's reagent was added. The test content was changed into white turbidity (or) precipitate which indicated the presence of alkaloids.

Steroids

The test extracts were treated with minimum quantity of chloroform, 3 to 4 drops of acetic anhydride and one drop of concentrated sulphuric acid. Purple color of the test content was changed into blue green. The result was qualitatively determined and recorded.

ANTIMICROBIAL ACTIVITY**Source of collection of plant material**

Capsicum frutescens L. and *Capsicum fastigiatum* L. was chosen as a plant sample for screening antibacterial activity. Small healthy leaves and stalks were collected during early hours of morning period from the fully grown plants at Thiruthangal, Virudhunagar District, and Tamil Nadu.

Processing of medicinal plants

The leaves and stalk collected were washed with water to remove soil and dust particles. Then they were dried in thoroughly shaded place, and blended to form a fine powder and stored in airtight containers.

Human pathogenic bacteria species

The human pathogenic bacteria such as *Pseudomonas*, *Enterobacter*, and *Escherichia coli* were obtained from Vivek Laboratory, Nagercoil, and Tamilnadu.

Preparation of plant extracts

The fresh leaves and stalk (10g) are taken and it is ground with 5% ethanol with the help of mortar & pestle. The extract was filtered with the help of muslin cloth.

Dry plant extract

The 5g of powder is taken and 25ml of ethanol is added and it is kept in a shaker for 2hrs. The extract was filtered with the help of muslin cloth.

Disc preparation:

What man No-1 filter paper was used to prepare disc with the help of punching machine. The discs were sterilized in autoclave and loaded with suitable concentration of test extracts.

Disc diffusion method

Filter paper disc diffusion technique in agar was followed to determine antimicrobial activity by the procedure of Garg and Jain, 1998. What man No.1 filter paper discs of 6-mm diameter, placed in dry petri plates, were autoclaved. Sterilized filter paper No.1 discs were loaded with the extracts of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. using different solvents. The amount of extracts loaded in each disc was in the concentration viz., 25 µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml. Similarly discs were prepared for standard antibiotic penicillin (w/v) and cefotaxime (w/v) and were impregnated in the filter paper discs in different concentrations 25 µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml.

The plates were incubated for 24 hrs at 37°C. After incubation the diameter of inhibitory zones formed.

3. RESULTS

In vitro antibacterial assay the efficacy of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. to inhibit the growth of pathogenic microbes showed the ethanol extract of the plants had broad spectrum of antibacterial potential. The phytochemistry and antimicrobial activity of *Capsicum frutescens* and *Capsicum fastigiatum* was analysed and recorded.

PHYTOCHEMICAL ACTIVITY

Preliminary screening of phytochemical content in *Capsicum frutescens* L. and *Capsicum fastigiatum* L. was carried out after extracting the plant with different solvents such as butanol, ethanol, and water. The extracts of the *Capsicum frutescens* L. and *Capsicum fastigiatum* L. were subjected to standard chemical test for the detection of different phytoconstituents. All the tested phytochemical were found to contain in the various extracts of the leaf of *Capsicum frutescens* L. and stalk of *Capsicum fastigiatum* L. were analysed and recorded in Table 1 and Table 2.

In *Capsicum frutescens* L. the ethanol extract containing remarkable positive results of phytochemical compared to other solvent extracts. The ethanol extract shows flavonoids, tannins and saponins, phenolic compounds, steroids, sugar and reducing sugar. In these compounds flavonoids and tannins have important antibacterial potential efficiency. (Table 1).

In *Capsicum fastigiatum* L. ethanol extract showed highest positive results of phytochemical compared to other solvents extracts. The ethanol extract shows alkaloids, tannins saponins, phenolic compounds, steroids and reducing sugar. In these compounds flavonoids and alkaloids have important antibacterial potential efficiency (Table 2).

Screening of anti bacterial activity

Antimicrobial screening of the leaf extracts were analysed in disc diffusion methods.

Disc diffusion method

All solvent extracts of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. used in the present study exhibited degrees of antibacterial activity against tested pathogenic microorganisms *Enterobacter sp.*, and *Pseudomonas aeruginosa*, when studied by the disc diffusion method. The ethanol extract of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. was found to be effective against all tested micro organisms with inhibition zone ranging with an average of 2.0 – 5.9 mm in extracts prepared by mortar and pestle extractions (Table 3). When the result was compared with standard antibiotics Penicillin and Cefotaxime a moderate antibacterial efficiency was observed in the ethanol, butanol and water extracts of *Capsicum frutescens* L. and *Capsicum fastigiatum* L..The standard antibiotics Penicillin and Cefotaxime inhibited the maximum growth of the test bacterial strains at the concentration of 100µg/ml.

The ethanol extract of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. showed highest inhibition activity against *E.coli* and *Enterobacter sp.*, around 5.4 ± 100 µg/ml (Table 3). In the present study, the ethanol extract of *Capsicum frutescens* and *Capsicum fastigiatum* shows maximum zone of inhibition against pathogenic test organisms when compare to other solvent extracts.

From the overall observations of the present investigation, among the various extracts, the ethanol extract of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. was found to have the highest activity than all other extracts for the tested pathogens. It is interesting to note that regarding minimum inhibitory concentration value of the various extracts of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. against tested pathogens, the ethanolic extract was found to inhibit good and that bacteria only at the concentration of 80, 60 µg/ml and thereby it wouldn't affect the beneficial micro flora of intestine. Therefore, it may be recommended for the preparation of plant based drugs against human bacterial pathogens.

Table 1. Preliminary phytochemical screening of leaves of *Capsicum frutescens* L. using different organic solvents.

S.No.	Test	Extracts		
		Butanol	Ethanol	Aqueous
1	Reducing sugar	-	+	+
2	Sugar	+	+	+
3	Alkaloids	-	-	+
4	Steroids	-	+	-
5	Phenolic compounds	+	+	+
6	Flavonoids	+	+	+
7	Tannins	-	+	-
8	Amino acids	+	-	-
9	Saponins	-	+	+

Table 2. Preliminary phytochemical screening of different extracts of the *Capsicum fastigiatum* L. using different organic solvents.

S.No	Test	Extracts		
		Butanol	Ethanol	Aqueous
1	Reducing sugar	-	+	+
2	Sugar	+	+	+
3	Alkaloids	+	+	+
4	Steroids	-	+	-
5	Phenolic compounds	+	+	+
6	Flavonoids	-	-	-
7	Tannins	-	+	+
8	Amino acids	+	-	-
9	Saponins	+	+	+

Table 3. The antibacterial effect of *Capsicum frutescens* L. and *Capsicum fastigiatum* L. extracts against human pathogenic bacterial organisms in disc diffusion method.

Solvent	Conc. (µg/ml)	<i>Pseudomonas aeruginosa</i>		<i>Enterobacter sp.,</i>	
		<i>Capsicum frutescens</i> L.	<i>Capsicum fastigiatum</i> L.	<i>Capsicum frutescens</i> L.	<i>Capsicum fastigiatum</i> L.
Butanol	25	3.1 ± 0	3.0±0.2	2.0± 0	2.2±0.14
	50	3.3±0.1	3.5±0.12	2.1±0.14	2.8±1
	75	3.4 ± 0	3.7±0	3.3± 0	3.2±0.15
	100	3.7 ± 0	3.9±0.1	4.0 ± 0	3.8±0
Ethanol	25	2.6±0.28	2.2±0.1	2.0 ± 0.1	2.1±0
	50	3.3 ± 0.1	2.9±0.14	3.0 ± 0	3.2±0.1
	75	4.4 ± 0.1	3.6±0	4.1 ± 0.2	3.8±0.14
	100	4.6±0.14	4.0±0.18	5.4± 0.14	4.2±0
Water	25	2.7±0.14	3.1±0.1	2.1± 0.24	2.4±0
	50	3.1±0.24	3.5±0.14	2.4± 0.24	3.0±0.14
	75	3.5±0.31	3.9±0	2.0 ± 0	3.8±0.1
	100	3.9±0.14	4.0±0.28	2.6± 0.28	2.0±0.15
Penicillin	25	4.0±1	4.2±0.12	3.0±1	3.2±0
	50	4.3±0	4.5±1	3.1±1	3.8±2
	75	4.4±1	4.7±0.1	4.3±0	4.8±1
	100	4.7±1	5.0±0	5.0±1	5.2±0.1
Cefotaxime	25	4.4±2	4.8±0.18	5.0±1	5.1±2
	50	4.8±1	5.0±0.1	5.2±0	5.3±1
	75	5.0±0	5.2±0	5.4±1	5.6±0.14
	100	5.2±1	5.7±2	5.8±2	5.9±0.28

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