



Comparative Effects of *Capsicum Frutescens* and *Capsicum annum* Extract on *Bacillus Licheniformis*, *Pseudomonas frederiksbergensis* and *Bacillus Gibsonii* Isolated from Packed Spices

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ABSTRACT

Chilli is common spice in consumption and it provides numerous health benefits, also used for long term storage of foods especially in packed foods. Chemical preservatives are common but nowadays people commonly prefer natural preservatives than chemical preservatives due to the consequences of the health. The *Capsicum frutescens* has some health beneficial properties like anti-cancer activity, reduction of blood cholesterol level, maintenance of blood pressure and reduction of blood thickness etc. Chilli includes significant amounts of pigments with possible health advantages, including as lutein, anthocyanin, and chlorophyll, as well as other exceptional chemical components that may be beneficial to health. Here the dried semi solid filtrate was prepared from fresh chilli extract. Uniform suspension of microorganisms was obtained. Isolation of organism from commercial chilli powder packs. The antibacterial activity of capsaicin of *Capsicum frutescens* and *Capsicum annum* against the pathogens isolated from the packed spices and compare the activity of both chillies. Contaminants were isolated from the commercially available packed spices and identified by microscopic and biochemical tests. A microbial colony from the cultured plate to be identified and confirmed is placed onto a MALDI "target" plate. The confirmation of the organisms done by MALDI-TOF technique. The contaminants were identified as *Bacillus licheniformis*, *Pseudomonas frederiksbergensis* and *Bacillus gibsonii*. Capsaicin of *Capsicum annum* did not show any antibacterial activity against the organisms but capsaicin of *Capsicum frutescens* shows antibacterial activity against the organisms. So we can use *Capsicum frutescens* as natural preservative for the chilli powders, since it has lot of health benefits and also contains wide range of nutritional components and pharmacologically active metabolites.

KEYWORDS: Capsaicin, Spice Contaminants, *Bacillus licheniformis*, *Pseudomonas frederiksbergensis*, *Bacillus gibsonii*

Received 22.10.2022

Revised 20.11.2022

Accepted 16.12.2022

INTRODUCTION

Spices are used all over the world to prepare foods, mainly because of their flavouring properties. Chilli is the one of the spices mostly used for cooking. Most of these are grown and harvested in warm, humid areas of the world, where the wide variety microorganisms present and it may be exposed to wide range of environmental microbial contamination during the harvesting and processing. In many spice-growing countries, spices are sundried by spreading on the tarfelt sheet on road or open field after harvest. After that sold without any treatment to less the microflora [1]. Some spices harbor more numbers of microorganisms including both spore forming bacteria and spoilage organisms. Sometimes the spices treating substances contains spoilage organisms and may even introduce harmful organisms. The bacteriological safety of spices is done by using different ways, the methods are limited by cost, time required to completely destroy microorganisms and also their effect on flavor and color of spices. Although gamma irradiation was found to be more effective to reducing the microbial population of spices [2].

Capsicum plants belongs to the family Solanaceae and kingdom Plantae consist of 27 species, and it contains approximately 3000 varieties. Chili peppers are used worldwide in food due to their pungent flavor, aroma, and to increase the shelf life of food. Capsicum is an important agricultural crop and one of the most popular vegetable because of the combination of color, taste and nutritional values of the fruit

not because of its economic value. The interest in the consumption of Capsicum is due to their content of bioactive compounds and their importance as dietary antioxidants. Pepper used as fresh, dried, fermented, or as an oleoresin extract. It has both nutritional and nutraceutical importance. It is used as a colourant, flavourant and as pungency. The chilli flavor is also important in many products in addition to its color. Dried chilli is also valued due to their contribution to flavor in chilli sauces and chilli powder. The flavoring principle is associated with volatile aromatic compounds and color. As a general believe that when the color of chilli powder fades, the flavor also disappears. The volatile and non-volatile substances contribute to its use as flavoring agent. *Capsicum frutescens* is used traditionally as an external therapy in areas of shoulder, arm and spine for painful muscle spasms. It also used for the treatment of diabetes, toothache, blood pressure (high/low), bronchitis, burning feet, mouth sores and infected wound, reduce blood clots and aid digestion by stimulating saliva and gastric juice flow. One of the major by-products produced during the processing of peppers is pepper seeds (*Capsicum annum* L.), which make about 45%–50% of the fruits. The pepper seed oil has antioxidant activity and is high in unsaturated fatty acids, including vitamin E, polyphenols, and carotenoids. This oil has bioactivity and a phytochemical makeup that make it a potential UV protectant for the cosmetics sector.

Capsicum is a multipurpose fruit, which shows some pharmacological and toxicological potential. Capsicum used as anti-inflammatory, anti-diabetic, anti-cancer, anti-ulcer, anticoagulant, analgesic, anti-arthritis, immuno-modulatory, memory enhancing, pain relief, hypocholesterolaemic, hypolipidemic, hepatoprotective and antimicrobial effects. It also cause irritation to mucous membrane, inflammation and neurotoxicity. It should not be used on open wounds or abrasions, or near the eyes. It has antagonistic effects on of α -adrenergic blockers, clonidine and methyldopa. Capsicum species can be eaten raw or cooked. Generally varieties of Capsicum species such as *Capsicum annum* and *Capsicum frutescens* are used for the cooking purposes. Those are contains wide range of nutritional components and pharmacologically active metabolites. Both are used for wide range of pharmacological activities. Several carotenoid pigments such as capsanthin, capsorubin, cryptoxanthin and zeaxanthin are gives red color to the matured pepper fruits. The carotenoid pigments are present as fatty acid esters. An alkaloid compound called capsaicinoids (CAPS) is produced by the fruit, which is main source of pungency [3].

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a chili pepper extract and is the active component of Capsicum plants. It is responsible for the 'burning' sensation of chillies. Capsaicin is a part of the secondary metabolite family 'Capsaicinoids' that has been known to evolve as a defense mechanism in plants against mammals. Which is used as food and medicinal purposes since ancient times, and it gives pungency of their fruit. It also have some other properties like pain relief, cancer prevention, and beneficial cardiovascular, and gastrointestinal effects. Capsaicin also shows antimicrobial and anti-virulence activity. It shows bactericidal effect against food-borne pathogens such as *Helicobacter pylori*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* [4-5].

Capsaicin used in low concentrations used to protected the gastric mucous of rats against ulceration caused by ethanol. Anticancer activity of capsaicin was recorded in different types of cancer; capsaicin blocked breast cancer cell migration and killed prostate cancer cells. Coatings and powders are used with varying degrees of success to deter browsing animals and insects, and break children's thumb sucking and nail biting [6]. Pepper spray, whose active ingredient capsaicin is used widely for personal protection, law enforcement, and defence [7].

Presence of *Bacillus licheniformis* is common in foods and usually regarded harmless. Meanwhile food poisoning cases and also toxin producing strains of *B. licheniformis* have been reported. *B. licheniformis* has also associated with septicemia, peritonitis, ophthalmitis, and food poisoning in humans, as well as with bovine toxemia and abortions. Food-borne *B. licheniformis* outbreaks are predominantly associated with cooked meats and vegetables [8]. *Pseudomonas frederiksbergensis* is a gram negative motile rods and strictly aerobes. Colonies are smooth and pale yellow. *Pseudomonas* species are very important decomposers of organic matters in water, soil and food products. They are also pathogenic to plants, animals and humans [9]. *Bacillus gibsonii* is a gram positive, facultative anaerobe. It is alkaliphilic and alkali tolerant, aerobic endospore-forming bacteria.

MATERIALS AND METHODS

Sample collection

Fresh chillies (*Capsicum annum*, *Capsicum frutescens*) were collected from malappuram, kerala.

Preparation of plant extracts

Fresh chillies were washed with water to remove impurities and sun dried for three weeks. 20 gm fine powder of both chillies (*Capsicum annum*, *Capsicum frutescens*) were mixed with 50 ml of 95% Ethanol. It is kept in the shaking incubator for 24 h. The solvents were filtered by using whatsmann no:1 filter paper

and the solvent poured in to the 50 ml of beaker for the evaporation of ethanol. The dried semi solid filtrate was collected and stored in the refrigerator for further experiments [10].

Isolation of organism from commercial chilli powder packs

Serial dilution & plating

One gram of chilli powder from the five types of commercially available chilli powder packets were added to a conical flask containing 100 ml of sterile distilled water and shaken vigorously to obtain a uniform suspension of microorganisms. This was labeled as the dilution of 10^{-1} . Serial dilution was performed following the method of Hedges, 2002. 0.1 ml of the diluted sample from each dilutions (10^{-2} to 10^{-7}) were transferred to their respective sterile plates by spread plate method. the plates were then incubated at 37°C for 24 hours. After incubation, plates were observed for the growth of bacteria [11].

Identification of the organism from commercial chilli powder packs

To identify the microbes isolated from the commercially available chilli powder packs, Gram's staining [12], Endospore staining [13], Motility test - Hanging drop method [14] and Biochemical test [15] that includes Indole test, Methyl Red test, Voges Proskauer test, Citrate Utilization test, Oxidase test, Catalase test, Urease test were performed.

Confirmation of identified organisms Isolated from chilli powder packs

MALDI-TOF - Matrix-assisted laser desorption/ionization time-of-flight [16]

A microbial colony from the cultured plate to be identified and confirmed is placed onto a MALDI "target" plate, treated with formic acid, embedded within matrix, and the laser is aimed at the target plate to begin sample collection. The detected biomolecules are not identified from the resultant spectrum; rather, the unique pattern produced by proteins and other biomolecules from the microorganism is matched to a spectrum of a known microorganism from a database, like fingerprints are matched to identify individuals. Each spectrum contains peaks unique to specific genera, species, and strains and the MALDI-TOF machine uses an algorithm to produce a value on the level of confidence in its identification.

Antibacterial activity of plant extract against organisms Isolated from chilli powder packs

Well diffusion

The plant extracts of both *Capsicum annum*, *Capsicum frutescens* was diluted with DMSO solution. The plates were prepared by Muller-Hinton agar medium and inoculated with overnight cultures of test organisms. Four wells were made in each plate by using well cutter. Different concentration of chilli extract solution added to the wells. Plates were incubated at 37°C for 24 hours and the zone of inhibition is measured [5].

RESULTS AND DISCUSSION

Extraction

The chilli extract of ethanol were obtained within 5 days of evaporation and the viscous extract collected was stored at 4°C in a sterile screw capped bottle and is maintained for further analysis (Fig. 1).

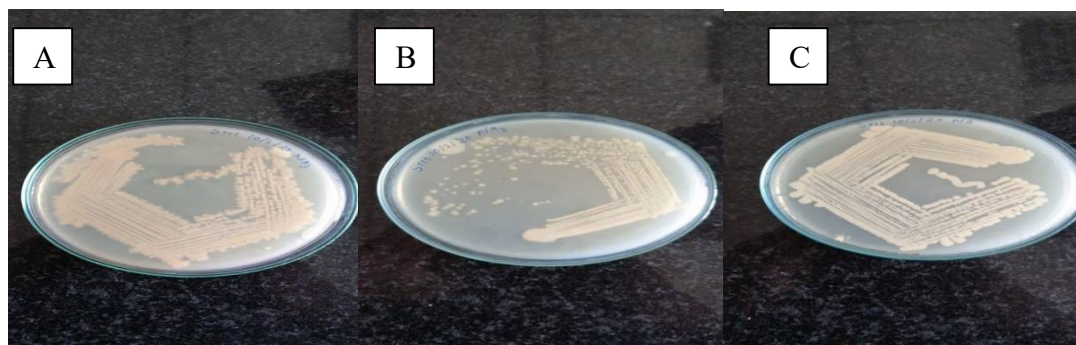
Fig. 1: Ethanol extraction of Chilli extract



Isolation of organisms

Three different organisms were isolated from the commercially available packed chilli powders were screened based on the cultural characteristics of colonies on nutrient agar plates (Fig. 2).

Fig. 2: Growth of A) Isolate 1, B) Isolate 2 and C) Isolate 3 in nutrient agar

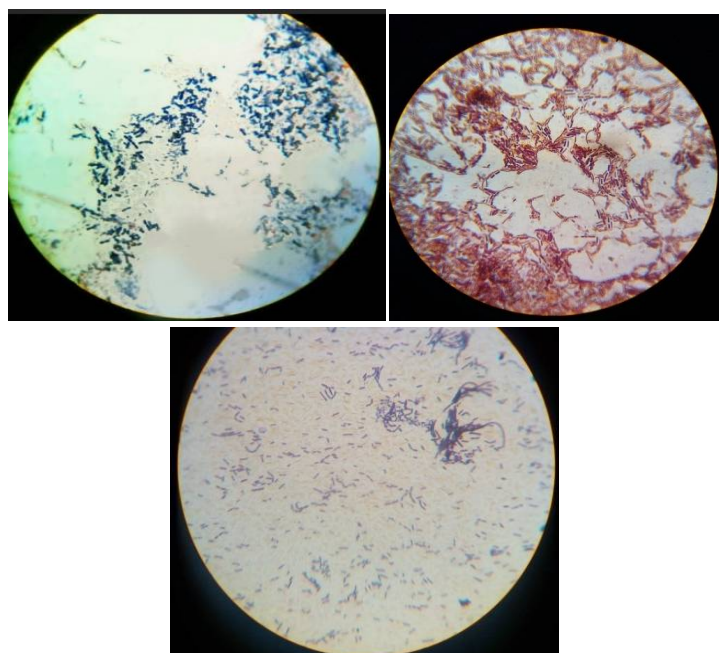


Identification of organisms

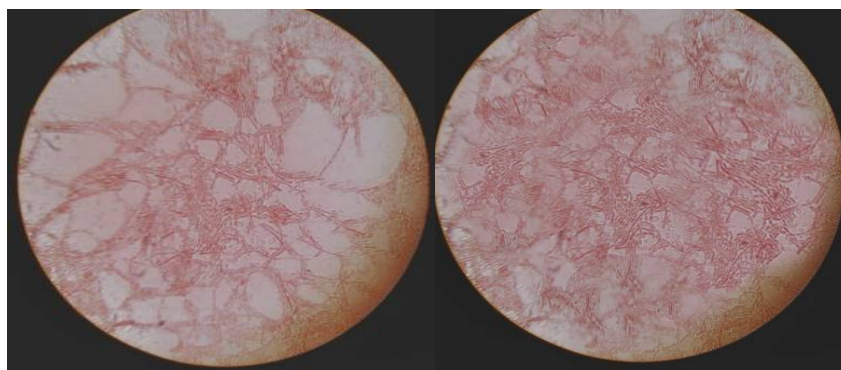
Isolated organisms were identified by using different microscopic and biochemical tests (Fig. 3& Fig. 4) and the results were recorded and tabulated in Table 1.

Table 1: Microscopic and biochemical identification of organisms

Tests performed	Isolate 1	Isolate 2	Isolate 3
Gram's staining	Gram positive	Gram negative	Gram positive
Endospore staining	Endospore forming	Non endospore forming	Endospore forming
Motility test	Motile	Motile	Motile
Indole test	-	-	-
Methyl red test	-	-	-
Voges Proskauer test	+	-	-
Citrate utilization test	+	+	-
Catalase test	+	+	-
Oxidase test	+	+	+
Urease test	-	-	-



**Fig. 3: Gram staining of Isolate 1,2 &3 respectively
Fig. 4: Endo spore staining of Isolate 1 &3 Respectively**



Confirmation of identified organisms

The identified organisms were confirmed as *Bacillus licheniformis*, *Pseudomonas frederiksbergensis* and *Bacillus gibsonii* by using MALDI-TOF technique.

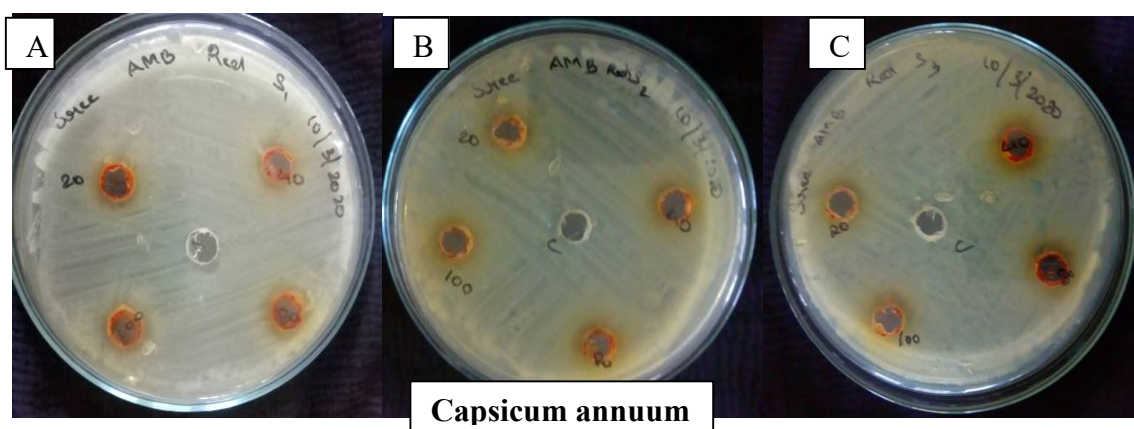
Antibacterial activity of plant extract - Well diffusion technique

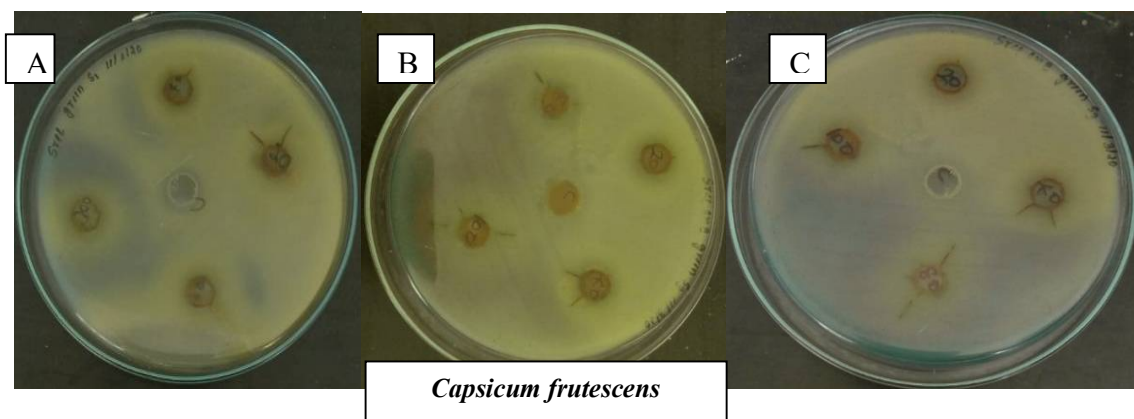
The antibacterial activity of *Capsicum annum* and *Capsicum frutescens* was performed in Muller Hinton agar plates through well diffusion method. The diameter of the clear zone around the wells containing plant extract against the three organisms were measured and tabulated. All three bacteria are showing increasing sensitivity along with increasing concentration against extract of *C. frutescens*. The zone diameters were recorded in tabulated (Table 6.2). *Capsicum annum* did not show any antibacterial activity against the isolated organisms such as *Bacillus licheniformis*, *Pseudomonas frederiksbergensis* and *Bacillus gibsonii*. Only *Pseudomonas frederiksbergensis* shows sensitive to 20µg/ml concentration of capsaicin of *Capsicum frutescens*. It shows higher zone diameter in 100µg/ml concentration of capsaicin. 100µg/ml showed maximum activity in the case of *Pseudomonas frederiksbergensis* and *Bacillus licheniformis*. 80µg/ml showed maximum activity in the case of *Bacillus gibsonii*.

Table 2: Antibacterial of plant extract

Sl.no	Organisms	Zone of inhibition in mm							
		Extract of <i>C. annum</i>				Extract of <i>C. frutescens</i>			
		20µg	40µg	80µg	100µg	20µg	40µg	80µg	100µg
1.	<i>Bacillus licheniformis</i>	0	0	0	0	0	8	10	14
2.	<i>Pseudomonas frederiksbergensis</i>	0	0	0	0	4	9	10	15
3.	<i>Bacillus gibsonii</i>	0	0	0	0	0	7	13	11

Fig. 5: Antibacterial activity of *Capsicum annum* & *Capsicum frutescens* against A) *B. licheniformis*, B) *P. frederiksbergensis* & C) *B. gibsonii*





Food poisoning is a concern of both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are still concern with the high and growing number of outbreaks caused by some food spoilage microorganisms. Consequently, they are using different ways to stop this upward trend and reduce the incidence of food poisoning. Spices and herbs have been used to food since ancient times, not only as flavoring agents. It also used as folk medicine and food preservatives. Increasing of antibiotic resistant microorganisms and increasing food consumers have to be using new and natural antimicrobials. One area of research is developing new and improved methods of food preservation. Additives are not good for human health that causes some health issues in human body. Spices worth over 100 million dollars are exported every year from India because of the popularity of Indian spices for their aroma and flavor. They are frequently reported to be highly contaminated with bacteria and molds. There is no data available based on the microbial quality of any spices sold in unpackaged form. Therefore, a survey was made to check the microbial quality of two spices, cumin seeds and chili powder obtained in unpackaged form and the effect of environmental conditions of storage and sale [18].

The frequent contamination by microorganisms in imported spices has been causing serious problems in the food industry in Japan. The contamination of spices may occur at the time of harvesting, handling, transportation and storage in the exporting countries. Processed spices with a high bio-burden utilized in the food industry accelerate the putrefaction of manufactured food products. Mold growth under unfavorable conditions occurs during long storage and shipment should also cause quality changes and occasionally mycotoxin production. In many countries using fumigation with ethylene oxide and heat sterilization, it has been tried with varying degrees of success. However, these methods have several disadvantages for application to the sterilization of spices such as toxic residues are left and organoleptic properties are changed [2].

CONCLUSION

The present study was carried out to evaluate the antimicrobial properties of *Capsicum frutescens* against the pathogens isolated from commercially available packed chilli powders. *Capsicum frutescens* has many health benefits such as external therapy in areas of shoulder, arm and spine for painful muscle spasms. It also used for the treatment of diabetes, toothache, bronchitis, burning feet, mouth sores and infected wound, reduce blood clots and aid digestion by stimulating saliva and gastric juice flow. It helps to maintain the blood pressure by reducing the thickness of blood cholesterol level. The isolated contaminants from commercial spice packs were *Bacillus licheniformis*, *Pseudomonas frederiksbergensis* and *Bacillus gibsonii*. The plant extract was prepared by using 95% ethanol. The antibacterial activity of the plant extract was done using disc diffusion method in Muller Hinton agar plates. The overall result indicates that the plant extract has good antibacterial activity. The antibacterial activity of the plant extract against the three organisms was different. 100µg/ml showed maximum activity in the case of *Pseudomonas frederiksbergensis* and *Bacillus licheniformis*. 80µg/ml showed maximum activity in the case of *Bacillus gibsonii*. The isolated organisms from the packed chillies show sensitive to capsaicin of *Capsicum frutescens*. Therefore we can use *Capsicum frutescens* as preservative for the chilli powders. By using *Capsicum frutescens* as a preservative agent we will get advantages like many health benefits.

ACKNOWLEDGEMENT:

The authors acknowledge Chancellor Shri A.Srinivasan, Dhanalakshmi Srinivasan Group of Institutions, for the financial support of this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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CITATION OF THIS ARTICLE

Anchana, V.V., Uma, Devi, Pongiya, Saranya, C, Saravanakumari.P and Sandeep Kumar Singh: Comparative Effects Of *Capsicum Frutescens* and *Capsicum Annuum* Extract On *Bacillus Licheniformis*, *Pseudomonas Frederiksbergensis* and *Bacillus gibsonii* Isolated From Packed Spices. *Bull. Env.Pharmacol. Life Sci., Spl Issue* [5]: 2022:592-598.