



ANTIBACTERIAL AND PHYTOCHEMICAL ANALYSIS OF *Capsicum annum* (chili pepper BSS-859 F₁ Hybrid) AGAINST FOODBORNE PATHOGENS

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Abstract: Currently used medications are monetarily costly and they are not expeditiously. Medicative plants contain enormous varieties of chemical substances that have necessary therapeutic properties that may be utilized in the treatment of human diseases. This study was conducted to determine the effect of crude extract and fractions of *Capsicum annum* on selected food borne pathogens (*Salmonella spp.*, *Staphylococcus aureus*, *Shigella*, *Bacillus cereus*). The methanolic extract of chili pepper (BSS-859 F₁ hybrid) showed good activity against *Staphylococcus aureus* and *Bacillus spp.* Compared to crude extract and four fractions from chili extract tested, chili pepper extract showed the greatest activity against gram positive and gram-negative enteric pathogens but the gram-negative bacteria showed less activity than gram positive bacteria.

Index terms – Antibacterial activity, *Capsicum annum*, Foodborne pathogens

I. INTRODUCTION

Food borne infections comprise a public health concern for developed and developing countries. Many developing countries use ancient medications as the essential health care level. Currently used drugs are financially costly and they are not instantly available. The conflicting utilization of those medications could prompt to microbial drug resistance. Medicative plants contain enormous varieties of chemical substances that have vital therapeutic properties that might be utilized within the treatment of human diseases. Foodborne pathogenic microorganisms present an immediate risk to public health. Universally, 2.2 million people die every year from diarrhea caused by contaminated food and water, including 700,000 diarrheal deaths.

Antimicrobial agents have consistently carried major clinical importance, particularly in this time of developing antibiotic resistance amongst common disease-causing pathogens. In a country like India where no clinical prescription needed to buy antibiotics, resistance to antibiotics is promoted significantly.

Chili pepper have a wide range of uses, including pharmaceutical, natural coloring agents and cosmetics as a decorative plant, and because the active ingredient in most defense repellents. In a review studies, the genus capsicum had been reported to possess analgesic, hypoglycemic, gastroprotective, anticancer, antimicrobial, anticholesteremic, antidiabetic, antiparasitic, anticlotting, antioxidant, antipyretic and larvicidal activities provide relief against rhinitis, sinusitis, migraine, diabetes and arthritis. Chili pepper contain capsaicin, is a phenolic compound.

As of now, foods are typically preserved by compound such as nitrite, sodium benzoate, and sodium meta bisulfide that have been tested and proven safe (Gould and Russell, 2003). Nonetheless, there are safe occasional reports of hypersensitive response to these preservatives and even potential formation of carcinogenic by products like nitrosamines from nitrite (Roller, 2003). Plants and herbs contain many various classes of phytochemicals include terpenoids, alkaloids, lectins, polypeptides, quinones, phenolics, flavonoids, coumarins and etc. (Cowan,1999).

In addition to the use of chili pepper as an ingredient, it is used in traditional medicine. Other than capsaicin's different pharmacological and physiological properties (pain relief, cancer prevention, beneficial cardiovascular, and gastrointestinal effects), it has recently attracted considerable attention due to its antimicrobial and anti-virulence activity. Bactericidal effect has been demonstrated against *Helicobacter pylori*, *Pseudomonas aeruginosa* (Cowan, 1999; Omolo *et al.*, 2014), whereas anti-virulence activity has been demonstrated against *Vibrio cholerae*, *Staphylococcus aureus*, and *Porphyromonas gingivalis* (Chatterjee *et al.*, 2010; kaila *et al.*, 2012; Qiu *et al.*, 2012; Zhou *et al.*, 2014). Most of the gastrointestinal diseases are as a result of contamination. It's

also used in ancient medications particularly treating symptoms such as stomach ache, diarrhea, dysentery. Foodborne gastroenteritis affects several million people yearly throughout the globe. Incidence of foodborne illness remains a serious problem, even in developed countries.

Extracts from capsicum annum fruit have been investigated to some extent antimicrobial properties have been reported with mixed results. Crude tissue extracts from several different *Capsicum annum* varieties have inhibited growth of species of *Bacillus*, *Clostridium*, *Pseudomonas*, *Listeria*, *Salmonella*, *Staphylococcus* (Bacon *et al.*, 2016).

II. MATERIALS AND METHODS

2.1 Collection of chili pepper: In the present study, chili pepper was collected based on their ethnic usage histories. The collected fruits were preserved for extract preparation to accomplish *in vitro* bioassays.

2.2 Extraction of chili pepper (J Das *et al.*, 2018): Dried chilies were prepared from ripened, red coloured chili. They were brought from a grocery store, Annur. The chilies were dried for 4 days to obtain dried chili and ground to a fine powder. Hexane and methanol were used to extract the chili pepper. About 30g of chili powder was extracted in 300 mL of organic solvents viz. hexane and methanol by Soxhlet extraction method. The extracts were evaporated by rotary vacuum evaporator to obtain crude extract. The extracts were stored in refrigerator for further qualitative assays and *in vitro* investigations.

2.3 Column chromatography: A suitable size long cylindrical glass column was stand on a column-chromatography stand. Silica gel was mixed with methanol and packed in cylindrical glass column. Then the PBS buffer were passed through column at uniform rate under gravity to fractionate the sample extract. Each fraction was collected separately in the test tube and numbered consecutively for further analysis.

2.4 FTIR analysis: Fourier Transform Infrared (FTIR) Spectroscopy is usually referred as **FTIR Analysis** or FTIR Spectroscopy. This infrared spectroscopy method is used to identify organic, polymeric, and in some cases, inorganic materials. The FTIR test relies on infrared light to scan samples and observe bond properties. FTIR analysis services can identify compounds and the general type of material being analysed when there are unknowns. This technique is used to assess the purity of some inorganic samples and is highly reliable for identifying polymer composition.

2.5 Phytochemical analysis of chili pepper (*Capsicum annum*) extract

The chili extract was tested for phytochemical constituents. Alkaloids, carbohydrates, saponins, terpenoids, steroids, phenol, tannins, flavonoids.

2.6 Antibacterial activity

The standard cultures *Staphylococcus aureus*, *Bacillus cereus*., *Salmonella spp.*, *Shigella* were procured from Bioline laboratory, Rs puram (Coimbatore). The bacterial strains were conformed for their purity and the pure cultures were sub cultured and incubated at 37°C for 18-24hrs. The subcultures were stored in refrigerator for further use. Sterile Muller- Hinton Agar (MHA) (g/L) (Beef infusion: 300; casein hydrolysate: 17.5; starch: 1.5; Agar: 15; pH: 7.2±0.2) was prepared. The media was sterilized at 121°C at 15 lbs for 15min. diluted bacterial cultures was spread evenly over the entire surface of the agar plates using sterile cotton swab. Using a well puncture 5 wells of 50µm diameter were made for drug loading. The test extracts were prepared and dissolved in DMSO. About 50µl of extracts and column chromatographic fractions were dispensed in each well on the lawn culture plates. The plates were allowed to stand on the laboratory bench for 1h to allow proper diffusion of the extracts into the media and incubated at 37°C for 18-24 hours. After incubation the halo zone of inhibition were measured in millimetres.

III. RESULT AND DISCUSSION

The phytochemical analysis of (chili pepper BSS-859) *Capsicum annum* revealed the presence of alkaloid, steroids, phenol, saponin and terpenoids.

The Graph (Chart.1,2,3,4) represents Fourier Transform of Infra-Red analysis for different fractions of chili pepper (BSS-859) extract. The composition of fractions contains the fraction1, fraction2, fraction3, fraction4. The main functional groups present in these materials are secondary amines, carbon dioxide, alkene, halo compounds, amine, aliphatic ether, aldehyde etc.

(Chili pepper BSS-859 F₁ hybrid) *Capsicum annum* shows higher antibacterial activity against some enteric pathogens. Among four fractions and crude extract, crude extract inhibited gram positive and partially inhibited gram-negative bacteria at a concentration of 50µg ml⁻¹. Gram negative bacterial cell walls have an outer membrane which makes them intrinsically resistant to certain antimicrobials either because they lack the target of the antimicrobials or the antimicrobials cannot get into the cytoplasm (Suffrendiniet *al.*, 2017). The present study showed that the gram-positive bacteria was more sensitive against chili pepper BSS-859 F₁ hybrid), which is similar to the study by Daligaet *al.*, (2007). This is due to the structural differences in the outer layer of bacteria (Diao *et al.*, 2014). In gram positive bacteria, chili pepper (BSS-859 F₁ hybrid) showed maximum activity (zone of inhibition: 20mm) against *S. aureus* and in gram negative bacteria, chili pepper (BSS-859 F₁ hybrid) showed maximum activity (zone of inhibition: 17mm) against *Salmonella spp.*, This result justified because it is expected that the Gram-positive bacteria are found to be more susceptible due to the presence of outer peptidoglycan layer only, which is not an effective permeability barrier (Bamoniri A *et al.*, 2010).

Fraction 2 and 3 inhibition zone was 4mm to 1 mm in all pathogens. Fraction 2 showed no zone of inhibition in *Shigella spp.*, among 4 four fractions, F1 and F4 showed no activity against all enteric pathogens. This study confirmed that, the chili pepper BSS-859 crude extract was effective than separated components of chili pepper BSS-859. This may due to the different chemical compound that responsible to the antibacterial activity (Nurjanah S *et al.*,2016).

The extract of chili pepper (BSS-859 F1 hybrid) showed the encouraging results against *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella*. These bacteria are responsible for diarrhea, dysentery and stomach ache. Dorantes *et al.* reported that the *capsicum* extracts had an inhibitory effect on *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria* and *Bacillus cereus*. The antibacterial activities of enteric bacteria observed in this study confirmed results found by Dorantes *et al.*

In this study, (BSS-859 F1 hybrid) chili exhibited antibacterial activities against some enteric pathogens. Among the enteric pathogens tested, *Staphylococcus aureus*, *Bacillus cereus* was the most sensitive compared to the other 3 organisms.

Compared to crude extract and four fractions tested, crude extract of (chili pepper BSS-859) *Capsicum annum* shows the higher antibacterial activity against some tested enteric pathogens.

FTIR analysis of *Capsicum annum* extract fractions

Chart.1 FTIR analysis of Fraction 1

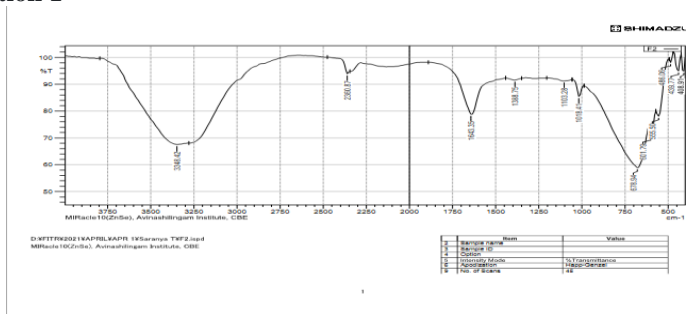


Chart 2. FTIR analysis of Fraction 2

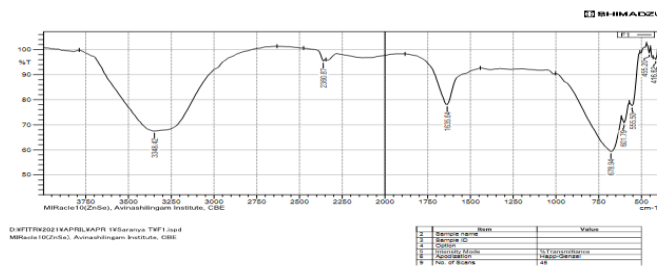


Chart.3 FTIR analysis of Fraction 3

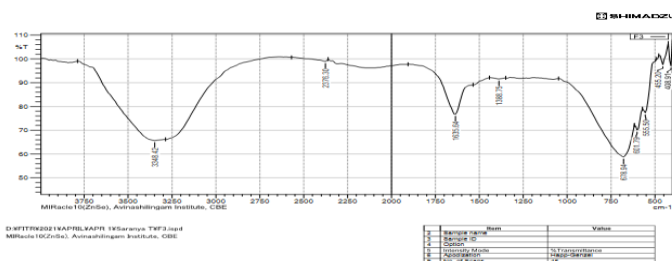


Chart.4 FTIR analysis of Fraction 4

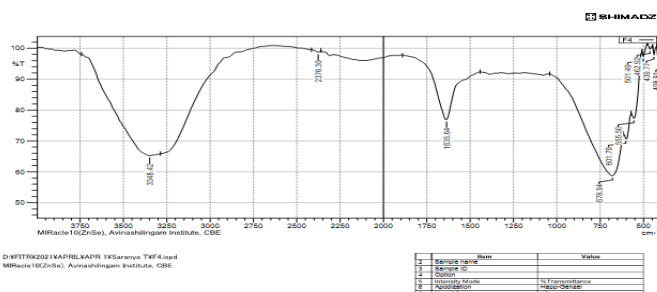


TABLE-1

DETERMIATION OF FTIR RESULTS

S.no	Fractions of crude extract	Functional groups	Wave number ranges
1.	Fraction 1	Secondary amines, carbon dioxide, alkene, halo compound	3348.42, 2360.87, 1635.64, 678.9, 601.79, 555.50, 455.20, 416.62
2.	Fraction 2	Secondary amines, carbon dioxide, Aliphatic ether, sulfoxide alkene/trisubstitute	3348.42,2360.87,1643.35,1388.75,1103.28,1018, 678,601,555.50,486.06,439.77,408.91
3.	Fraction 3	Secondary amines, alkene/trisubstitute, amine, halo compound.	3348.42,2376.30,1635.64,1388.75,678.94,601.79,555.50,455.20,408.91
4.	Fraction 4	Secondary amines, alkene, phenol, halo compound	3348.42,2376.30,1635.64,678.94,601.79,555.50,501.49,462.92,439.77, 408.91

TABLE-2

Antibacterial activity of (chili pepper (BSS-859) F₁ hybrid) *Capsicum annuum* against the selected enteric pathogens

S.NO	Name of the enteric pathogens	Antibacterial activity of chili pepper extract (BSS-859 F ₁ hybrid)	F1	F2	F3	F4
1	<i>Bacillus spp.</i> ,	18mm	-	1mm	1.5mm	-
2	<i>Shigella spp.</i> ,	8 mm	-	-	1mm	-
3	<i>Salmonella spp.</i> ,	16mm	-	1mm	1mm	-
4	<i>Staphylococcus aureus</i>	20mm	-	4mm	2mm	-

Figure.1 Screening of antibacterial activity of (BSS-859 chili pepper) against enteric pathogens:

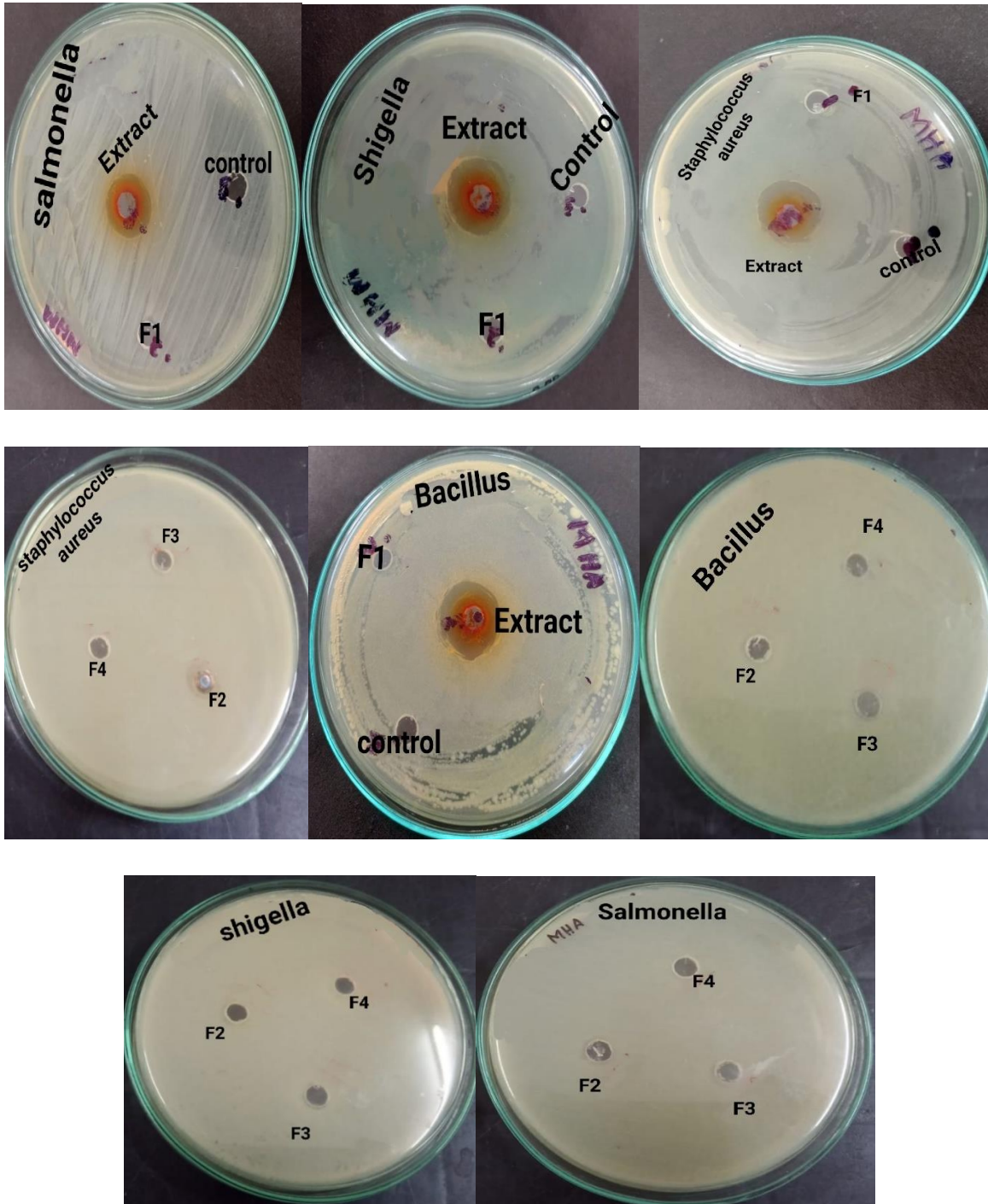


Figure.1

Figure1: shows the antibacterial activity of crude extract and fraction 1,2,3,4 of chili pepper BSS-859 against enteric pathogens

SUMMARY AND CONCLUSION: The present study was carried out to study the antibacterial activity of chili pepper (BSS-859 F₁ hybrid). The chili pepper (BSS-859) was collected based on their ethnic usage histories from a grocery shop, Annur. The chili peppers were shade dried and made into a powder form. The powdered chili peppers were subjected to Soxhlet extraction by using methanol as a solvent. The crude extract was tested for phytochemical analysis. The phytochemical screening revealed that the chili pepper contains alkaloids, phenols, saponins, steroids and terpenoids. The collected crude extract was subjected to column chromatography and the fractions were collected. The four fractions collected from column chromatography were tested in FTIR (Fourier Infra-Red spectroscopy). Among the four fractions tested, all fractions contain secondary amines. Then the crude extract and four fractions were tested for the antibacterial activity. Chili pepper (BSS-859 F₁ hybrid) extract was tested for antibacterial activity against the enteric pathogens such as *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Bacillus cereus*. The methanolic extract of chili pepper (BSS-859 F₁ hybrid) showed good activity against *Staphylococcus aureus* and *Bacillus cereus*. Compared to crude extract and four fractions from chili extract tested, chili pepper extract shows the greatest activity against gram positive and gram-negative enteric pathogens but the gram-negative bacteria showed less activity than gram positive bacteria.

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