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Comparative Study on Antimicrobial Activity of Eight Capsicum Species- A novel Therapeutic compound

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ABSTRACT

Infections caused by pathogens such as bacteria, fungi and protozoa can be prevented, managed and treated through antipathogenic group of compound known as antibiotics. The present investigation is to the identification of the broad spectrum antimicrobial activity of a total of eight species of Capsicum and its vertices such as Capsicum annuum. var. Chile Mulato, Capsicum chinense, Capsicum annuum var. glabriusculum, Capsicum hybrid- Pepper, Capsicum sp. var. Long slim Cayenne Hot Pepper, Birds Eye Chilli- Kanthari Mulaku, Capsicum annuum var. annuum, Capsicum. sp. Violet Kandhari Chili against two human pathogens Staphylococcus aureus and Streptococcus mutant. Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Among the samples, ethanolic extract of Capsicum annuum var. glabriusculum, Capsicum sp. var. Long slim Cayenne Hot Pepper and Capsicum. sp. Violet Kandhari Chili were shown high inhibition activity for two pathogens means of forming zone when compare to other Capsicum species. This comparative study on antimicrobial activity of capsicum species leads to development of effective antibacterial compound.

KEYWORDS Capsicum, Antibacterial, Staphylococcus aureus and Streptococcus mutant

Introduction:

Antimicrobial properties of the natural drugs were notable alternative to modern antibiotic drugs. Since the recent clinical report suggest that most of the microorganisms were developing resistance to many commonly used antibiotics because of the improper protocol of antibiotics prescriptions (1). The recent scientific community makes use of the early process of the Indian traditional system of medicine phenomena of endogenous or exogenous use of plant as the source of medicinal agent to cure many diseases (2). Moreover the over dosage of antibiotics will leads to severe diverse effect such as hypersensitivity, immunosuppression and allergic reactions. The report cited by the World Health Organization (WHO) estimate that 80% of the world population follows the traditional based therapies using of plant extracts and their phyto components (3).

Solanaceae belong to the family Nightshade Family which is consist of 2000 species among 90 genera distributed in the tropical and temperate regions of the world. In India, the family is represented by 15 genera and 88 species which are cultivated throughout out in Himalayas and Southern and Eastern parts. These families are of considerable economic importance and are a source of food (4). The solanaceae family is consider as the economically important plant family and ranks the first in terms of vegetable crops (1). The most common commercially cultivated species are potato (Solanum tuberosum), Egg plant (Solanum melongena), Tomato (Lycopersicum esculentum) Raspberry (Physalis peruviana), Chillies (Capsicum annum), Tobacco (Nicotiana tabacum) and Datura (Datura stramonium).

The genus capsicum annum which is commonly represented as chilli pepper well known species of flowering plant in solanaceae family. The origin of the capsicum species were from the south of Mexico. It was reported that, nearly 20 capsicum species are distributed worldwide. Among those, *Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum pendulum* and *Capsicum pubescens*. There are several genetically modified verities of these species were also commercially available and the exact estimation of the species and its verities were still controversial (2).

The present investigation is to the identification of the broad spectrum antimicrobial activity of a total of eight species of Capsicum and its vertices such as *Capsicum annuum. var. Chile Mulato*, *Capsicum chinense* , *Capsicum annuum var. glabriusculum*, *Capsicum hybrid- Pepper*, *Capsicum sp. var. Long slim Cayenne Hot Pepper*, *Birds Eye Chilli-* Kanthari Mulaku, *Capsicum annuum var. annuum*, *Capsicum. sp.* Violet Kandhari Chili selected on the basis of medicinal folklore reports and their common use in Indian traditional systems of medicine.

Materials and Methods:

Plant material

The eight species of Capsicum were collected from different places of the kanyakumari district, Tamilnadu, India and further species identification was carried out by the Department of Botany, S.T.Hindu College, Nagercoil, Kanyakumari District (Table 1).

Preparation of plant extracts

The 20 g portions of the powdered plant material were soaked separately in 100 ml of ethanol for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatman filter paper no. 1 (Whatman, England). The filtrates obtained were concentrated in vacuum using rotary evaporator at 30°C.

Species	Plant	Leaf & Flower	Fruit
Capsicum annuum. var. Chile Mulato			

Capsicum chinense - hybrid Grande			
Capsicum annuum var. glabriusculum			
Capsicum hybrid- Pepper			
Capsicum sp. var. LONG SLIM CAYENNE Hot Pepper			
BIRDS EYE CHILLI-KANTHARI MULAKU			
Capsicum annuum var. annuum			
Capsicum. sp. Violet Kandhari Chili			

Table 1: Collected Plant Species and their profile

Microbial samples
The microbial strains used are *Staphylococcus aureus* (SA) and *Streptococcus mutant* (SM) were collected from Vivek laboratory, Nagercoil, Kanyakumari district, Tamilnadu.

Maintenance of microorganisms
The test bacteria's were maintained in nutrient agar slants. The microbial cultures were subcultured and the cultured strains were allowed to grow 24 hours at 37ºC, stored at 5°C for further studies.

Inoculums
The bacteria were inoculated into soyabean casein digest agar : broth and incubated at 37°C for 4 h and the suspension were checked to provide approximately 105 CFU:ml.

Preparation of sample extract for microbiological assay
About 1gm of plant extract was dissolved in 10 ml (100 mg / ml-1) of DMSO to obtain a stock solution. The plant extract was diluted as 1:10 equivalent to 100 mg/ml-1 and 1:5 dilution equivalents to 50 mg/ml-1.

Antibacterial assay by disc diffusion method
Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Bacterial strains were swabbed using sterile cotton swabs in nutrient agar plate. Disc of 6 mm were punched from Whatman No.1 filter paper. Up to 10 µl of each concentration of the extract were respectively introduced in the discs using sterile pipettes. The disc was then placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters (Bauer *et al*., 1966).

Results:
Disc method of crude Ethanolic extract:
Present study on Antimicrobial activity ethanolic extract of eight Capsicum sample leaf, stem and fruit use disc method against two human pathogenic bacteria such as SA and SM. Among the samples, ethanolic extract of *Capsicum annuum var. glabriusculum*, *Capsicum sp. var. Long slim Cayenne Hot Pepper* and *Capsicum. sp. Violet Kandhari Chili* were shown high inhibition activity for two pathogens means of forming zone. Ethanolic extract of *Capsicum hybrid- Pepper* and *Capsicum sp. var. Long slim Cayenne Hot Pepper* showed medium activity against the two pathogens and the negative inhibition zones were predicted for *Capsicum annuum. var. Chile Mulato* and *Capsicum chinense* (Table 2) (Figure 1). The *Capsicum annuum. var. Chile Mulato* leaf, stem and fruit showed no activity zone formation against the SA and SM pathogens. The *Capsicum chinense* no zone formation observed against SA. *Capsicum chinense* fruit showed (10mm) followed by stem (9mm) and leaf have no activity against SM (Figure 1).

The *Capsicum annuum var. glabriusculum* leaf and stem showed (12mm) and fruit (10mm) activity zone against SA. The *Capsicum annuum var. glabriusculum* leaf showed (11mm) followed by stem (8mm) and no activity in fruit against SM. The *Capsicum hybrid- Pepper* fruit showed (11mm) followed by leaf (8mm) and stem (8mm) activity against SA. The *Capsicum hybrid- Pepper* observed no activity against in SM. The *Capsicum sp. var. Long slim Cayenne Hot Pepper* fruit showed (11mm) followed by stem (10mm) and leaf (8mm) activity against SA. The *Capsicum sp. var. Long slim Cayenne Hot pepper leaf* showed (12mm) followed by stem (8mm) and no activity in fruit against SM (Figure 1).

The *Birds Eye Chilli-* Kanthari Mulaku leaf showed (10mm) followed by fruit (9mm) and stem (8mm) activity against SA. The *Birds Eye Chilli-* Kanthari Mulaku leaf, stem and fruit showed no zone formation against in SM. The *Capsicum annuum var. annuum* fruit showed (11mm) followed by leaf (8mm) and no activity observed in stem against SA. The *Capsicum annuum var. annuum* fruit observed (9mm) and no activity in leaf and stem against SM. The *Capsicum. sp.* Violet Kandhari Chili fruit showed (13mm) followed by stem (11mm) and leaf (8mm) activity observed against in SA. The *Capsicum. sp.* Violet Kandhari Chili leaf showed (14mm) followed by fruit (12mm) and stem against no activity showed in SM (Figure 1).

Discussion
Infectious diseases represent a serious health problem today and major cause of one third of death worldwide. Infections caused by microorganisms such as bacteria, fungi and protozoa can be prevented, managed and treated through antibacterial group of compound known as antibiotics (5). Antibiotics are one of the most important weapons in fighting against bacterial infection and have greatly benefited to human welfare since the day of its introduction. The wide use of antibiotics in the treatment of bacterial infection has lead to emergence and spread of resistant strains (6). Increasing development of drug resistance human pathogens as well as the side effect of synthetic drugs urges us to discover new antimicrobial drugs from natural sources. This situation has forced to search new antimicrobial substances from various sources like medicinal plant, fungi etc (7, 8). Among the sources, plants have enormous therapeutic potential which are effective in the treatment of infectious diseases (8). Plant extracts are active against drug resistant pathogens because of the presence of bioactive compounds which target different binding sites (9-11).

The compounds such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds in the plants serves as a defense mechanism against microorganisms, insects and other herbivores (12). Therefore scientific attention were towards phytomedicines and biologically active compounds isolated from plant which are therapeutic index for the development of novel drugs (13, 14). The present study shows the antibacterial efficiency of *Capscicum* is evaluated against two gram positive bacteria *Staphylococcus aureus* and *Streptococcus mutants*.

The stem and fruit extract of *Capsicum annuum var. glabriusculum* and *Capsicum sp. var. Long slim Cayenne Hot Pepper* shows highest activity of against *Staphylococcus aureus* followed by fruit extract of *Capsicum. sp. Violet Kandhari Chili* and the leaf extract of *Capsicum annuum var. glabriusculum* and *Capsicum. sp.* Violet Kandhari Chili shown maximum zone inhibition respectively. The leaf extract of sample *Capsicum hybrid- Pepper*, *Capsicum annuum var. annuum* and *Capsicum. sp.* Violet Kandhari Chili fruit extract produce high inhibition zone against *Staphylococcus aureus*. The fruit and leaf extract shows highest activity compare to standard antibiotics the photochemical present in the Capsicum fruit may be the reason for that.

The leaf extract of *Capsicum. sp.* Violet Kandhari Chili shows highest value of inhibition zone against *Streptococcus mutants*. Whereas, the *Capsicum. sp.* Violet Kandhari Chili fruit extract and the stem extract of *Capsicum sp. var. Long slim Cayenne Hot Pepper* shows moderate activity against the bacteria *Streptococcus mutants*. The fruit and leaf extract shows highest activity compare to standard antibiotics the photochemical present in the Capsicum fruit may be the reason behind (Figure 2, 3). Thus the overall outcome resembles the possible of developing an effective and low cost antimicrobial drug and it will post the formers as well the economic importance for the country.

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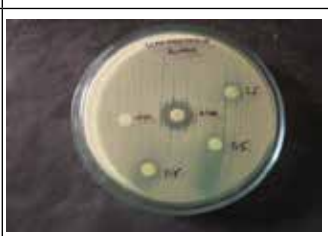
Table 2: Antimicrobaial Properties of Capsicum species against Staphylococcus aureus and Streptococcus mutants.

S. no	Plant	**Staphylococcus aureus** (inhibition zone formation in mm)			**Sterptococcus mutans** (inhibition zone formation in mm)			**Control** (inhibition zone formation in mm)			
		Leaf	Stem	Fruit	Leaf	Stem	Fruit	Positive		Negative	
1	Capsicum annuum. var. Chile Mulato	NZ	NZ	NZ	NZ	NZ	NZ	20	27	NZ	NZ

S. no	Plant	**Staphylococcus aureus** (inhibition zone formation in mm)			**Sterptococcus mutans** (inhibition zone formation in mm)			**Control** (inhibition zone formation in mm)			
		Leaf	Stem	Fruit	Leaf	Stem	Fruit	Positive		Negative	
2	Capsicum chinense	NZ	NZ	NZ	NZ	9	10	20	23	NZ	NZ
3	Capsicum annuum var. glabriusculumv	12	12	10	11	8	NZ	20	25	NZ	NZ
4	Capsicum hybrid- Pepper	8	8	11	NZ	NZ	NZ	19	24	NZ	NZ
5	Capsicum sp. var. Long slim Cayenne Hot Pepper	8	10	11	12	8	NZ	18	25	NZ	NZ
6	*Birds Eye Chilli*- Kanthari Mulaku	10	8	9	NZ	NZ	NZ	20	25	NZ	NZ
7	Capsicum annuum var. annuum	NZ	8	11	NZ	NZ	9	20	24	NZ	NZ
8	*Capsicum. sp.* Violet Kandhari Chili	8	11	13	14	NZ	12	19	25	NZ	NZ

NZ (no zone)

S.no	Plant	Staphylococcus aureus	Sterptococcus mutans
1	***Capsicum annuum. var. Chile Mulato***		
2	***Capsicum chinense***		
3	***Capsicum annuum var. glabriusculumv***		
4	***Capsicum hybrid- Pepper***		
5	***Capsicum sp. var. Long slim Cayenne Hot Pepper***		

6	***Birds Eye Chilli*- Kanthari Mulaku**		
7	***Capsicum annuum var. annuum***		
8	***Capsicum. sp.* Violet Kandhari Chili**		

Figure 1: Antimicrobaial Properties of Capsicum species against *Staphylococcus aureus* **and** *Streptococcus mutants***.**

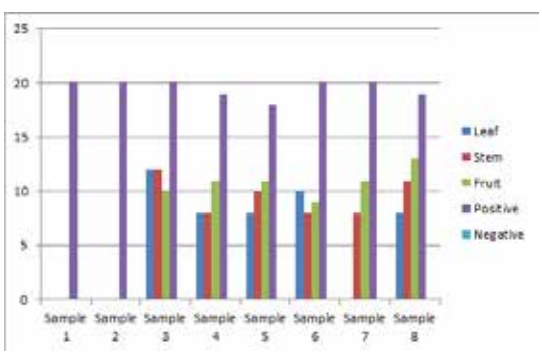


Figure 2: Antibacterial efficiency of *Capscicum* **species evaluated against** *Staphylococcus aureus*

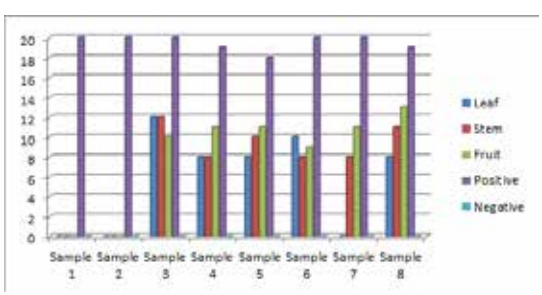


Figure 3: Antibacterial efficiency of *Capscicum* **species evaluated against** *Streptococcus mutants*

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