

Original Research Article

In Vitro Analysis of the Antibacterial Property of Chili (*Capsicum frutescens*) to Methicillin Resistant *Staphylococcus aureus*

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Abstract: This study aimed to evaluate the antibacterial property of chili (*Capsicum frutescens*) to Methicillin Resistant *Staphylococcus aureus*. Specifically it aimed to: 1. Determine the effect of different concentrations of Chili (*Capsicum frutescens*) extracts to MRSA in vitro. 2. Determine the relationship between concentrations of extracts to their antibacterial property: a. Pure extract b. 75% extract c. 50% extract d. 25% extract. 3. Compare the effectiveness of Chili (*Capsicum frutescens*) extracts with Vancomycin as a positive control. Pearson Correlation Analysis was conducted to examine whether there is a relationship between concentrations of extracts to their antibacterial property. Based on the findings of the study, the following conclusions were drawn as (a) MRSA is susceptible to 100% Chili (*Capsicum frutescens*) extract, intermediate to 75% Chili (*Capsicum frutescens*) extract and is resistant to 50% and 25% Chili (*Capsicum frutescens*) extract. (b) Higher percentages of concentration of extract were associated with higher antibacterial property. All different concentrations of Chili (*Capsicum frutescens*) extract are significantly weaker in inhibiting the growth of MRSA as compared with Vancomycin.

Keywords: Antibiotics, Capsaicin, Intermediate, Pathogen, Resistant, Susceptible, Zone of inhibition.

INTRODUCTION

Microorganisms can be both beneficial and a threat to humans. Those that cause diseases are called pathogens. Pathogens include: Bacterium, a group of microscopic organisms that are capable of reproducing on their own, causing human disease by direct invasion of body tissues. Bacteria often produce toxins that poison the cells they have invaded [1].

These microorganisms need complete eradication, inhibition, or if not reduction to a level that is under ecological balance. The most common way of inhibiting the growth of bacteria is the use of antibiotics. Antibiotics are medicines used to treat infections or diseases caused by bacteria. They work by blocking vital processes in bacteria, killing the bacteria, or stopping them from multiplying. This helps the body's natural immune system to fight the bacterial infection. Antibiotics have saved millions of lives since they were first introduced. However, because they have been overused, many antibiotics are no longer effective against the bacteria they once killed.

A growing concern of medical practice today lies on the ability of some pathogenic bacteria to withstand the effects of established antibiotics. Factors such as overuse and misuse of antibiotics, unsound industrial utilization of pharmaceuticals, livestock feeding with antibiotics and household use of

antibacterial soaps and other products contribute to the overwhelming increase in resistance of pathogens to antibiotics. This leaves some bacteria alive which, for some certain reasons, undergo mutation. The mutated bacteria multiply again passing the mutation to their progenies. In this way, bacteria evolve into a new drug-resistant strain. Other than this mechanism, the artificial implantation of "resistance gene" can also induce drug-resistance [2].

Staphylococcus aureus is one of the most common bacteria isolated from humans. It is a common etiologic agent of many skin infections. These bacteria exert its activities by extruding a range of toxins including alpha-toxin, beta-toxin and the like. Consequently *Staphylococcus aureus* is included in the list of drug resistant bacteria. Most strains were once sensitive to penicillin but due to repeated utilization, the bacteria developed a defence mechanism against it. Aside from penicillin it developed resistance too to antibiotics such as cephalosporin, methicillin, dicloxacillin, nafcillin and oxacillin. Thus the term MRSA (Methicillin Resistant *Staphylococcus aureus*) was coined.

With the emergence of *Staphylococcus aureus*' resistance to broad spectrum of antibiotics, the search for natural antibiotics to fight this drug resistant bacteria has become a phenomenon. The use of herbal drugs

known to provide safe and natural alternative treatment for many health problems were explored to investigate their antibacterial properties.

One plant that possess characteristic value for experimental study is Chilispecifically *Capsicum frutescens*. This specific genus of Chilli contains capsaicin. Capsaicin proved to be a compound with a large bioactivity with manifested actions over the cardiovascular, respiratory and nervous systems and with a good antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus cereus*, *Listeria monocitogene*, *Helicobacter pylori* [3]. According to Mori, A. et.al.[4], when capsaicin was taken orally, the substance almost caused 80% of cancer cells to suicide in lab mice and prostate tumors treated with capsaicin were about one-fifth the size of those in untreated mice. Other medicinal purposeinvolve pain relief. According to R. Bryson [5], a topical form of capsaicin is a recognized treatment for osteoarthritis pain, and may also help alleviate pain from long-term nerve damage caused by diabetes (diabetic neuropathy). Capsaicin is also a potent anti-inflammatory agent. According toJ. Lokesh [6],it works by inhibiting Substance P, which is associated with inflammatory processes. Capsaicin is being studied as a potential treatment for sensory nerve fiber disorders, including pain associated with arthritis and psoriasis. It also has the ability to lower the risk for diabetes. Study made by Bartolome, DJ et. Al[7]also proved that Chili (*Capsicum frutescens*) has antibacterial property against *Staphylococcus aureus*, however its effect to MRSA is not yet established thus, this study was conducted.

Statement of the Objective

The study aimed to evaluate the antibacterial property of chilli (*Cpsicum frutescens*) to Methicillin Resistant *Staphylococcus aureus*.

Specifically it aimed to:

1. Determine the effect of different concentrations of Chilli (*Capsicum frutescens*) extracts to MRSA in vitro.
2. Determine the relationship between concentration of extracts to their antibacterial property:
 - a. Pure extract
 - b. 75% extract
 - c. 50% extract
 - d. 25% extract
3. Compare the effectiveness of Chili (*Capsicum frutescens*) extracts with Vancomycin as a positive control.

MATERIALS AND METHODS

The following were the materials/ intruments used in the study: CHILI (*Capsicum frutescens*) fruits, gauze pad, sterile cotton swabs, inoculating loop, what man filter paper, plastic bag, funnel, graduated cylinder, beakers, Erlenmeyer flask, culture tubes, stirring rod,

petri dishes, spatula, verniercaliper, autoclave, weighing scale, incubator, mechanical grinder, hot air sterilizer, Mueller-Hinton agar, Nutrient agar, distilled water, Methicillin Resistant *Staphylococcus aureus*.

Research Design

Pearson Correlation Analysis was conducted to examine whether there is a relationship between concentrations of extracts to their antibacterial property.

Preparation of Culture Media for Subculture

Nutrient agar media was prepared from a commercial dehydrated base according to manufacturer's procedure. The Nutrient agar was weighed, placed in an Erlenmeyer flask and dissolved by adding the appropriate amount of distilled water. The mixture was boiled with continuous stirring until it was clear. After complete dissolution of the agar, it was autoclaved and allowed to cool at room temperature. The media was then poured to culture tubes and positioned in a slanting manner until it solidified.

Inoculation of Test organism (MRSA): Sub-culturing

The pure culture of MRSA was inoculated in the Nutrient agar slant medium. Using sterile loop, the inoculum was deposited at the bottom of the NA slant medium and streaked in a zigzag manner towards the mouth of the tube. After inoculation, the media was incubated at 37°C for 18 hours for the organism to grow.

Preparation of Culture Broth

Pure colonies of MRSA grown in the NA slant medium were transferred to Nutrient broth medium to achieve turbidity comparable to 0.5 McFarland standards.

Preparation of Mueller-Hinton Agar

Mueller-Hinton Agar was prepared from a commercially available dehydrated base according to the manufacturer's procedure. After autoclaving it was allowed to cool and was poured to sterile petri dishes to achieve a uniform depth of approximately four (4) mm. The agar medium was allowed to cool and solidify at room temperature.

Preparation of Paper Discs

Paper discs approximately six (6) mm in diameter were prepared from Whatman filter paper no.1. The paper discs were prepared by punching the filter paper using clean and sterile puncher and were place in a petri dish. To ensure that the disc were sterile they were autoclaved and dried in a hot air oven.

Collection of Chili (*Capsicum frutescens*) Fruit Crude Extract

Fruits of *Capsicum frutescens*, specifically the ripe ones were collected washed with water and blot dried.

The fruits, using blender, were grinded and squeezed using sterile gauze to obtain the crude extract.

To make 75% Extract, 7.5 ml of the crude extract was mixed with 2.5 ml of distilled water. 50% extract was obtained by mixing 5.0 ml of crude extract and 5 ml of distilled water. 25% extract was prepared by mixing 2.5 ml of crude extract and 7.5 ml of distilled water.

Inoculation of Test Plates

A sterile cotton swab was dipped into the adjusted suspension of the nutrient broth. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum on the swab. The Mueller-Hinton agar plate is inoculated by streaking the swab over the entire agar surface evenly. The inoculated plates were allowed to dry for five minutes.

Application of Disc to Inoculated Agar Plates

The discs soaked in the different extract concentrations were inoculated into the surface of the seeded agar plate. Each disc was pressed down to ensure complete contact with the agar. The discs were placed not closer than 24 mm from the center and 15

mm away from the edge of the petri dish. The plates were inverted and incubated for 24 hours. This procedure was done for three consecutive days (three replicates).

Measurement of Zone of Inhibition

Zones of inhibition implicated by the different treatments, if any, were measured. The clear portion surrounding the disc was measured using Vernier calliper.

Zone of inhibition measuring ≥ 13 mm is susceptible, zone of inhibition measuring 11-12 mm is intermediate, zone of inhibition measuring ≤ 10 is resistant.

Resistant - indicates that clinical efficacy has not been reliable in treatment studies. Intermediate – implies clinical applicability in body sites where the drug is physiologically concentrated or when a high dosage of the drug can be used. Susceptible - implies that an infection due to the organism may be treated with the concentration of antimicrobial agent used, unless otherwise contraindicated[8].

RESULTS AND DISCUSSION

Table 1: The mean Zone of Inhibition of the different concentration of Chili (*Capsicum frutescens*) extracts to MRSA in vitro.

Concentration extract	Mean	SD
100% (pure)	13.17	1.1005
75%	12.85	0.1670
50%	9.42	0.8287
25%	9.36	0.9675

Table 1 shows the mean Zone of Inhibition of the different concentration of Chili (*Capsicum frutescens*) extracts to MRSA in vitro. It can be viewed from the table that the average concentration extract of pure (100%) extract is 13.17mm with standard deviation of 1.1005mm while for 75% extract the mean zone of inhibition is 12.85mm with standard deviation of 0.1670. A concentration extract of 50% has an average mean zone of inhibition of 9.42mm with a standard deviation of 0.8287 while 25% extract has an average mean zone of inhibition of 9.36mm with standard deviation of 0.9675. It can be concluded from the findings that the higher the percentage of

concentration extract the higher the zone of inhibition. Statistically, 75% concentration extract is the most accurate greater number of bacterial killings (SD = 0.1670) out of the four experimental groups.

Based on the result, MRSA is susceptible to 100% Chili (*Capsicum frutescens*) extract probably due to the Capsaicin content of the extract as supported by the studies conducted by Donnerer, J., et al. and Bartolome, DJ., et. al. MRSA is intermediate to 75% Chili (*Capsicum frutescens*) extract while it is resistant to 50% and 25% Chili (*Capsicum frutescens*) extract.

Table 2: Correlation between concentrations of extracts to their antibacterial property

r-value	p-value	Decision
0.956*	0.044	Significant

*significant

Table 2 shows a Pearson correlation analysis was conducted to examine whether there is a relationship between concentrations of extracts to their antibacterial property. The results revealed a significant

and high positive relationship (r = 0.956, N = 4, p = 0.044). The correlation is very high in strength. It can be concluded from the findings that the higher the percentage of concentration of extract the higher the

zone of inhibition. Higher percentages of concentration of extract were associated with higher antibacterial

property.

Table 3: Comparison on the effectiveness of Chili (*Capsicum frutescens*) extracts with Vancomycin as a positive control.

Concentration extract (experimental)		Vancomycin(control group)	
	Mean		Mean
100% (pure)	13.17	C1	30
75%	12.85	C2	30
50%	9.42	C3	30
25%	9.36	C4	30
Grand Mean	11.36	Grand Mean	30

Table 4: Paired samples test on the effectiveness of Chili (*Capsicum frutescens*) extracts with Vancomycin as a positive control.

t-value	df	p-value	Decision
-17.95	3	.000	Significant

Table 4 describes paired samples test information to ascertain whether there is a significant difference when we compare the effectiveness of CHILI extract with Vancomycin. The test revealed a significant difference between concentration extract and the control group ($t = -17.95$, $df = 3$, $p < .01$). The control group (Vancomycin) reported significantly higher zone of inhibitions (Mean = 30, SD = 0) than that of the experimental group (concentration of extract) (Mean = 11.36, SD = 0.7659). (see table 3).

The result suggests that all different concentrations of Chili (*Capsicum frutescens*) extract are significantly weaker in inhibiting the growth of MRSA as compared with Vancomycin.

CONCLUSIONS

Based on the findings of the study, the following conclusions were drawn:

1. MRSA is susceptible to 100% Chili (*Capsicum frutescens*) extract, intermediate to 75% Chili (*Capsicum frutescens*) extract and is resistant to 50% and 25% Chili (*Capsicum frutescens*) extract.
2. Higher percentages of concentration of extract were associated with higher antibacterial property.
3. All different concentrations of Chili (*Capsicum frutescens*) extract are significantly weaker in inhibiting the growth of MRSA as compared with Vancomycin.

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