International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 05 Issue 08 August 2022
DOI: 10.47191/ijcsrr/V5-i8-49, Impact Factor: 5.995
IJCSRR @ 2022
www.ijcsrr.org

The Efficiency of Capsaicin in Chilli on Antibacterial Activity of *Salmonella*

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ABSTRACT
This research aimed to study the extraction of capsaicin from *Capsicum annuum* and *Capsicum frutescens* fruits by the reflux extraction method and its ability to inhibit the growth of *salmonella* bacteria.
Material and method: Capsaicin from *Capsicum annuum* and *Capsicum frutescens* fruits were extracted by reflux method using acetone as a solvent. Then, the acetone was separated from the extracts by a rotary evaporator, taken the extracts for measure the absorbance and tested for inhibiting the growth of *Salmonella* bacteria. Each group of 3 samples were prepared. The control group is a 100µL solution containing *Salmonella* at a concentration of 1:1000, mixed with 900 µL of saline. Experimental groups were the plates mixed with *Salmonella* and the extracts. All plates were incubated for 24 hours at 35.5 °C. and counted bacterial colonies as the number of bacteria colonies forming units (CFUs).
Results: Revealed the light absorbance of the extracts from the *Capsicum annuum* and *Capsicum frutescens* was in the same range as the absorbance of pure capsaicin. To test the ability to inhibit the growth of *Salmonella* bacteria, the average number of *Salmonella* colonies was 134 CFUs in the control group, and the experimental groups with the capsaicin from both chilli peppers did not find the number of *Salmonella* colonies.
Conclusion: The extracts of *Capsicum annuum* and *Capsicum frutescens* fruits had capsaicin. The substances obtained from the extracts of both peppers were able to inhibit the growth of *Salmonella* with no difference in effectiveness.

KEYWORDS: Capsaicin, Reflux method, *Salmonella*.

INTRODUCTION

Diarrhoea is a significant health problem in Thailand. Diarrhoea is a symptom, not a disease. It represents the end product of multiple gastrointestinal dysfunctions. The definition of diarrhoea has traditionally been based on the volume, frequency, and consistency of stools. Patients often consider increased fluidity of stool as diarrhoea.[1] *Salmonella enterica* is a critical global cause of foodborne diarrhoea, with *Salmonella* Typhimurium (S.Tm) as one of the main serotypes affecting humans. S.Tm also serves as a prototype for studying the general principles of *Salmonella* infection biology.[2] Multiple enteropathogens, including *Shigella* and *Salmonella*, were widespread drug resistance in most world regions.[3] The author wants to study natural extracts that are cheap, easy to find, and have antimicrobial properties. That is used as a replacement for antibiotics to treat diarrhoea, which are expensive and have a chance of drug resistance. In Thai people's daily lives, various chillies are utilized to flavour various foods. Prik Khe-nu(*Capsicum annuum*) and Prik Chi-fa (*Capsicum frutescens* fruits)are the most commonly planted species.[4] Oleoresin capsicum (OC) is the natural lipophilic irritant and the pungent principle of capsaicinoids present in capsicum fruits. OC is an oily, deep red coloured mixture of many compounds, mainly composed of capsaicin (C), dihydrocapsaicin (DHC), nordihy- drocapsaicin (n-DHC), homocapsaicin (h-C), and homodihy- drocapsaicin (h-DHC)[5,6]. Capsaicin and dihydrocapsaicin contribute to around 80-90% of the full pungency in most chilli peppers.[7] Capsaicin has been employed in the past for its analgesic purposes.[8] Due to its characteristic pungent nature, it has attracted interest in antimicrobial studies.[9,10] Although the antimicrobial properties of capsaicin have been studied to inhibit the growth of some bacteria.
However, studies on *Salmonella* bacteria found conflicting results.[11,12,13,14] Therefore, the author is interested in studying the extract of capsaicin from Thai chillis, Khe-nu(*Capsicum annuum)*and Chi-fa (*Capsicum frutescens* fruits) by the reflux method and the antimicrobial activity of the extracts in inhibiting the growth of *Salmonella* bacteria.

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MATERIAL AND METHOD

Chillies Preparation and extraction

1000 grams of *Capsicum frutescens* fruits and *Capsicum annuum* were bought from Hatyai fresh market. The fruits were dried in a hot air oven at 65 °C for 24 hrs. and cut into small pieces with a food blender. After that 50 grams of each sample was extracted by using the reflux method at 56.5 °C for 5 hours and used acetone as a solvent. After extraction, the extracts were evaporated under a rotary vacuum evaporator at 57 °C to separate the acetone solvent. Then the extracts were analyzed by the UV-Visible spectrophotometer to determine the chain form of capsaicin and tested for antimicrobial activity.

Bacteria culture preparation

Salmonella bacteria were cultivated in a test tube with a liquid nutrient medium at 37 °C for 24 hours to increase the number of bacteria, then serial dilutions in phosphate-buffered saline were made the next morning, diluted at 1:1000.

Testing for antibacterial characteristics

Each group of 3 samples were prepared. For the control group, pipette out 100 µL of a 1:1000 solution containing *salmonella*, mix with 900 µL of saline in a test tube and disseminate over an agar medium in a glass plate using the spread plate method. Experiment group 1 got 100 µL of a 1:1000 concentration of *salmonella* combined with 900 µL of *Capsicum frutescens* extract. Group 2 was given 100 µL of a 1:1000 solution containing *salmonella* and 900 µL of *Capsicum annuum* extract. All plates were incubated for 24 hours at 35.5 °C. Then *Salmonella* colonies were counted as CFU units.

RESULTS

The findings of the absorbance measurements of compounds isolated from *Capsicum annuum*

The spectral lines from the absorbance measurements are uneven in the wavelength range of 200-328 281 nanometres (nm). That may be several compounds with different absorbance values. Measurements of 328-728 nm in the wavelength range showed smooth lines. That may be only one compound in the 328-728 nm range with absorbance in this wavelength range or containing many compounds but with the same absorbance in this range.

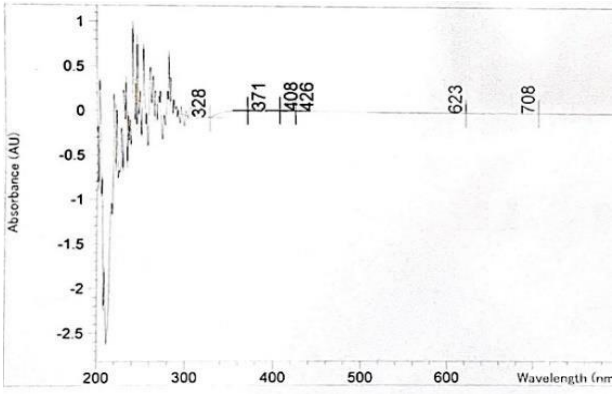

Figure 1: The absorbance measurements of compounds isolated from *Capsicum annuum*

The results of the measurement of absorbance from *Capsicum frutescens* extract.

In the 200-400 nm wavelength range, the spectral lines from the absorbance measurements are uneven. That might be the presence of several compounds with different absorbance values. The spectral lines in the 400-795 nm wavelength range were smooth. That might be the presence of only one compound with absorbance in this wavelength range or containing many compounds but with the same absorbance.

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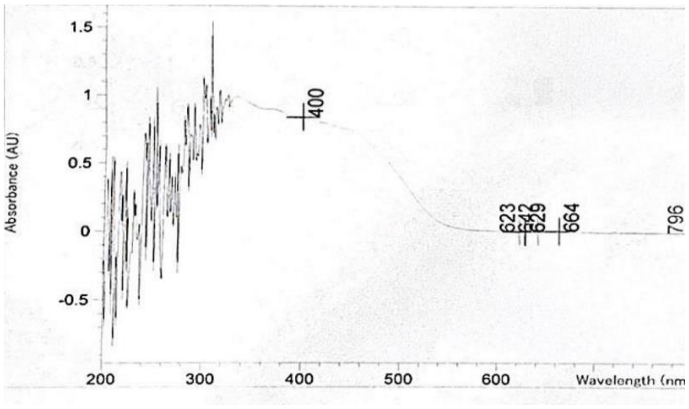

Figure 2: The absorbance measurements of compounds isolated from *Capsicum frutescens*

The study of capsaicin extract from *Capsicum frutescens* fruits, and *Capsicum annuum* inhibits *Salmonella* bacteria.

Samples		Number of bacterial colonies (CFU)
Control 100 µL of a solution containing *salmonella* at a concentration of 1:1000, mix with 900 µL of saline	Plate No. 1	113
	Plate No. 2	144
	Plate No. 3	145
Group 1 100 µL of a solution containing *salmonella* at a concentration of 1:1000, combined with 900 µL of *Capsicum frutescens* extract	Plate No. 4	0
	Plate No. 5	0
	Plate No. 6	0
Group 2 100 µL of a solution containing *salmonella* at a 1:1000 concentration along with 900 l µL of Capsicum annuum extract	Plate No. 7	0
	Plate No. 8	0
	Plate No. 9	0

Table 1 shows the *salmonella* colony count(CFU) results after inoculation at 35 °C for 24 hours.
The study results in the experimental process using extracts from *Capsicum frutescens* fruits and *Capsicum annuum* to test the ability to inhibit the growth of *Salmonella* bacteria found that Plate 1-3 control group, the development of salmonella in all three plates averaged 134 CFU. In experiment groups, Plate 4-6, the extracts from *Capsicum frutescens* fruits inhibit *Salmonella* bacteria's growth as the extracts from *Capsicum annuum*. Figures 3,4,5

Figures 3*: Salmonella* colonies in the control group

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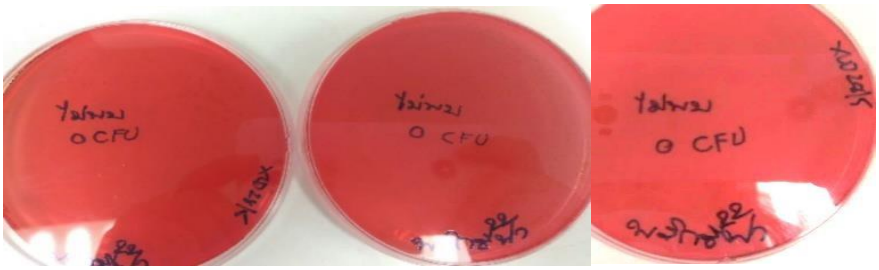

Figures 4: No *Salmonella* colony in the extract from *Capsicum frutescens* fruits

Figures 5: No *Salmonella* colony in the extract from *Capsicum annuum*

DISCUSSION

Measurement of light absorbance from the extracts of *Capsicum frutescens fruits* and *Capsicum annuum* has a value in a relatively wide range, about 200-795 nm, as shown in Figures 1 and 2. This effect may be due to many compounds in chilli peppers. Furthermore, reflux extraction methods cannot get pure capsaicin. Previous studies[15,16,17] found that pure capsaicin had an absorbance at a wavelength of 227-281 nm, and this study found that the absorbance was in the range of 200-300 nm, which is the same range as pure capsaicin. Therefore, the results of this study show that capsaicin is present in the extracts from *Capsicum frutescens* fruits and *Capsicum annuum*. Beyond the wavelengths of pure capsaicin, which is in the spectral range greater than 300 nm. the authors think it is a colourant in chilli peppers. Because of previous studies found that the pigments in chilli peppers absorb light at a wavelength of 475-500 nm, which is the same range as found in the experiment. Using extracts from *Capsicum frutescens* fruits and *Capsicum annuum* to test the ability to inhibit the growth of *Salmonella* bacteria, the author found that in the control group, the growth of *Salmonella* in all three plates averaged 134 CFU. Both experimental groups that combined extracts from *Capsicum frutescens* fruits and *Capsicum annuum* did not find any number of colonies of *Salmonella*. These results show that the capsaicin extracts from *Capsicum frutescens* fruits and *Capsicum annuum* can inhibit the growth of *Salmonella*. Acetone is an organic solvent. It is a substance that evaporates easily and quickly and it can destroy *Salmonella* bacteria. The author was aware of this effect, so the experimental process used a rotary evaporator to evaporate the acetone solvent from the extracts from both peppers. The evaporation process of the solvent by this method can evaporate almost all of the acetone or more than 99 per cent, making the extract from chilli the highest concentration, and there was no acetone remaining in the extract. The authors think other substances with light absorbance at a wavelength greater than 300 nm may be a colourant in chilli peppers because previous studies have found that the colourants in chilli absorb light at wavelengths in the range of 475-500 nm. The colouring agents in chilli do not inhibit the growth of *Salmonella*. Therefore, extracts obtained from *Capsicum frutescens* fruits and *Capsicum* annuum from this experiment have absorbance in the wavelength range of pure capsaicin, probably the capsaicin in the extracts and can inhibit the growth of *Salmonella*.

CONCLUSION

A UV-Visible spectrophotometer revealed that extracts from Khe-nu (*Capsicum annuum*) and Chi-fa (*Capsicum frutescens*) fruits contained capsaicin. The absorbance values were in the 200-300 nm range, and compounds derived from the extracts had the same ability to suppress *Salmonella* growth.

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Cite this Article: Supassara Peeyananjarassri, Panyapat Srisawat, Sumethinee Duanjamrun (2022). The Efficiency of Capsaicin in Chilli on Antibacterial Activity of Salmonella. International Journal of Current Science Research and Review, 5(8), 3206-3210