RESEARCH ARTICLE

Antimicrobial Activity and Cytotoxicity of "Bang Chang" Thai Cultivar Chili Pepper (*Capsicum annuum* Var. *acuminatum*)

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ABSTRACT

Capsaicin is found naturally in the Solanaceae family of plants and linked to numerous health advantage. Capsaicin is also responsible for the antimicrobial properties of chili pepper. Thai Capsicum cultivar "Bang Chang chili pepper" (*Capsicum annuum* var. *acuminatum*), initially cultivated in Bang Chang subdistrict, Samut Songkhram, Thailand. This study aims to determine bioactive substances such as capsaicin and phenolic content, as well as antimicrobial activity against pathogenic bacteria, *Staphylococcus aureus, S. epidermidis, Escherichia coli* and *Cutibacterium acnes*) and yeast, *Candida albicans*, and cytotoxicity with human skin fibroblast cells. The TPC and capsaicin in the ethanol extract were 2.50 ± 0.13 mg GAE/g and 0.0104 ± 0.0 mg/100 mL, while in the oil extract were 0.0020 ± 0.0 mg/100 mL and 1.05 ± 0.05 mg GAE/g. Antimicrobial of this chili pepper was found in only oil extract that was inhibited against to *C. albicans* (inhibition zone = 10.68 ± 0.49 mm) There was preferrable when compared to fluconazole ((inhibition zone = 24.65 ± 0.25 mm). Both extracts (0.0001-1.0 mg/mL) had no effect on human fibroblast cells, implying that they are non-toxic. The finding may imply that non-pungent capsicum strains cannot inhibit bacterial growth due to low amount of phenolics and capsaicin contained. Capsicum variety and temperature of extraction were also affected on their property. As a result, oil extract was favored for *C. albicans* suppression. This pepper extract can be used as an antifungal agent, and a pharmaceutical formulation must be developed.

Keywords: *Capsicum annuum var. acuminatum,* Chili pepper, Antimicrobial, Cytotoxicity.

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INTRODUCTION

According to the Food and Drug Administration (FDA), spices are the dried and aromatic portions of plants i.e., in whole, broken, or ground form, which are significant function in food is seasoning rather than nutrition. The primary distinction between herbs and spices is that spices can be derived from any part of plant rather than leaves, whereas herbs are usually derived from leaves. Spices are typically derived from dried plant parts such as buds, flowers (cloves, saffron), bark (cinnamon), root (ginger, turmeric), fruits/berries (cloves, chili, black pepper), or seeds (cumin), which contain volatile oils or aromatic aromas and flavors.[1, 2] For millennia, herbs and spices have played essential roles as flavoring agents, food preservatives, and medications. Many herbs and spices are known to possess qualities associated with lowering the chance of acquiring chronic diseases, therefore, research into their health benefits has risen dramatically over the last few decades. In particular, herbs and spices can protect against cardiovascular disease, neurological diseases, chronic inflammation, cancer, obesity, and type 2 diabetes. In addition, a variety of herbs and spices have also been recognized for their powerful antioxidant, antimicrobial, and anti-inflammatory properties.[3-10] Antimicrobial, including antibacterial, antiviral, and antifungal effects, which are also known for polyphenols, terpenoids, and other spice-derived alkaloids (such as capsaicinoids). This is one of the reasons why herbs and spices are commonly applied as food preservatives.[11, 12]

The chili pepper is a fruit spice obtained from Capsicum plants. The five domesticated pepper species are *Capsicum annuum, C. frutescens, C. chinense, C. pubescens,* and *C. baccatum*. Chili peppers have a pungent, fiery, and sweet flavor that varies based on the type and variety. Mild or sweet peppers have similar ingredients to capsicum but less or no noxious components. Chili peppers are used as food coloring, flavoring,

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predator repellants, and pain relievers. Capsaicinoids are the compounds responsible for the "hot" flavor of chili peppers, with capsaicin being the most well-known. Capsaicin is found naturally in the Solanaceae family of plants. It is widely used in both food and medicine, although its high pungency limits the amount that can be used. Capsaicin and capsinoids have been linked to numerous health advantages, including anticancer, anti-inflammatory, and analgesic properties.[13-20] Capsaicin is also responsible for antimicrobial properties of chili pepper. Several studies have suggested that chili pepper extracts, particularly capsaicin, exhibit antimicrobial effects against various microorganisms, including bacteria, fungi, and even some viruses. Capsaicin has antibacterial properties against a variety of bacteria, including some pathogenic strains. It can impair the integrity of the bacterial cell membrane, resulting in cell death. Studies have shown capsaicin to prevent the development of bacteria.[21, 22, 23] Chili pepper extracts have also been shown to have antifungal effects, including *Candida* species, which is appears to damage fungal cell membranes and impair metabolic activities.[24] Thai Capsicum cultivar "Bang Chang chili pepper" (*C. annuum* var. *acuminatum*), initially cultivated in Bang Chang subdistrict, Samut Songkhram, Thailand. Nutritional value and antioxidant activity have both been documented.[25, 26] This study aims to determine bioactive substances such as capsaicin and phenolic content, as well as antimicrobial activity against pathogenic bacteria and yeast, and cytotoxicity with human skin fibroblast cells, *in-vitro*. The discovery can be used or developed as a food preservative and skincare products without harmful effects.

MATERIALS AND METHODS

Plant Collection and Extraction

The seeds for the "Bang Chang" cultivar chili pepper (*C. annuum* var. *acuminatum*) were provided by the Tropical Vegetable Research Center at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, and were then planted in the Samut Songkhram Campus of Suan Sunandha Rajabhat University, Thailand, as the original area, from November 2022 to February 2023. Plant identification has already been validated.[25, 26] Harvesting red peppers or ripe fruits resulted in sun-dried chili peppers. Sun-dried chili peppers were regarded of appropriate quality because their moisture content was less than 1%. Fruit pedicels were removed, fruits were separated, and the remaining material was powdered. To extract the components, 100 g of chili pepper powder were macerated in 500 mL of pure ethanol. According to the oil extract, one liter of rice bran oil (RBO) was macerated with 500 g of chili pepper powder for three days. Each extract was filtered for contaminants, evaporated to a consistent weight, and stored at 4°C in an amber glass bottle.

Determination of Bioactive Compounds

Capsaicin

Capsicum extract was resolved in water (mobile phase) with 70% v/v acetonitrile, adjusted to 10.0 mg/mL concentration, and filtered before transferring sample container. Standard capsaicin (Sigma-Aldrich, USA) was made in the same manner as the sample, and the stock solution contained 1.0 mg/mL. The following conditions were used in the chromatography: mobile phase: 70% v/v acetonitrile in water; column: ACE Generix 5 C18 (4.6 250 mm, 5); injection volume: 20 l; flow rate: 1.0 mg/mL; detector: deuterium lamp 280 nm. A photodiode array detector portrayed the signal as a chromatogram corresponding to standard capsaicin. Capsaicin was maintained for around 5.0 minutes. The sample's capsaicin quantity was assessed by comparing it to a standard. Scoville heat units (SHU), a pungency measurement, were approximations.[27]

Total phenolic content

The Folin-Ciocalteu test was used to assess total phenolic content (TPC). The extract was dissolved in DMSO and combined with the Folin-Ciocalteu reagent and an appropriate alkaline solution. The blue hue produced was proportionate to the total phenolic content and was measured with a spectrophotometer at 760 nm. The extract was measured in milligrams of gallic acid equivalents per gram (mg GAE/g).[28]

Antimicrobial Test

Pathogenic bacteria and yeast

Pathogenic bacteria such as *Staphylococcus aureus, S. epidermidis, Escherichia coli* and *Cutibacterium acnes* were provided by Chiang Mai University's Faculty of Medicine, and pathogenic yeast such as *Candida albicans* were provided by Thailand's Institute of Scientific and Technological Research (TISTR), Pathum Thani.

Culture media antibiotic discs and equipment

As bacterial culture media, brain heart infusion, BHI (HiMedia Laboratories, India) was used. As yeast culture media, potato dextrose agar, PDA (HiMedia Laboratories, India) was employed. This test used a 6 mm filter paper disc (Macherey-Nagel, Germany), petri dishes (Union Science, Thailand), a laminar flow biohazard class II (Renovation Technology, Thailand), and a soft incubator SLIO-600ND (EYELA, Japan). The positive control antibiotic for *S. aureus* and *S. epidermidis* was 0.015 mg of erythromycin disc (Oxoid, UK). Positive control antibiotic for *C. acnes* was 0.002 mg of clindamycin disc (Oxoid, UK). Positive control antibiotic for *E. coli* was 0.01 mg of ampicillin disc (Oxoid, UK). For *C. albicans*, 0.025 mg fluconazole (Oxoid, UK) was used.

Disc diffusion method

In BHI and PDA plates, each bacterium and yeast were inoculated. Undiluted ethanolic extract (1 g) was dissolved in 95% ethanol (sterile with 0.2 μm membrane filtration) to concentrate at 100 mg/mL for disc diffusion. On the plate, 10 μL of undiluted extract was put onto a filter paper disc. The extract was then diluted (100 mg/mL) and applied to the disc 1, 2, and 3 times, at concentrations of 0.1, 1.0 and 10 mg, respectively. The tested disc was compared to a regular antibiotic disc and a negative control disc (95% ethanol) within the same plate. The incubation temperature was 37 ± 1°C for 24 to 48 hours. The test's interpretation was determined

by measuring the diameter of the inhibition zone (mm) that surrounding each disc. All steps of the test were carried out in an aseptic manner.[29]

Cell toxicity test

Each capsicum extract was dissolved in Dulbecco's modified Eagle's medium (DMEM), which contained DMSO (10%), FBS (10%), penicillin/streptomycin (1%), and sterile culture medium (0.0001, 0.001, 0.01, 0.1, and 1.0 mg/mL). Each sample or control well was cultured for 48 hours with suspended human skin fibroblasts (2.2–3.3 104 cells/mL). The cytotoxicity of viable skin cells was assessed by sulforhodamine B staining. Cell viability (%) of human skin fibroblast cells against SM was described as a result of four-time repeated studies. The positive (cytotoxic) control was sodium lauryl sulfate, while the negative (non-toxic) control was DMEM.[30]

Statistical Analysis

Descriptive statistics were utilized to represent and compare the bioactive content, antimicrobial activity, and anti-cytotoxicity of "Bang Chang chili pepper" to controls.

Table 1: Total phenolic content, capsaicin content and spiciness of capsicum extracts

Sample	*TPC* [a]	*Capsaicin*	*Pungency* [b]
(units)	GAE/g	mg/100 g	SHU
Ethanol extract	2.50 ± 0.13	0.0104 ± 0.0	0-700
Oil extract [c]	1.05 ± 0.05	0.0020 ± 0.0	0-700

[a] Total phenolic content (TPC) was mg of gallic acid equivalent (GAE) per g; [b] pungency (spiciness) of chili *peppers* and other substances, recorded in Scoville heat units (*SHU*); [c] Chili pepper were extracted with rice bran oil

Table 2: Antimicrobial activity of capsicum extracts*

Pathogen	*Extract/Control*	*Diameter of inhibition zone (mm)*			
		100 mg (undiluted)	*10 mg*	*1.0 mg*	*0.1 mg*
S. aureus	Ethanol extract	ND	ND	ND	ND
	Oil extract	ND	ND	ND	ND
S. epidermidis	Ethanol extract	ND	ND	ND	ND
	Oil extract	ND	ND	ND	ND
C. acnes	Ethanol extract	ND	ND	ND	ND
	Oil extract	ND	ND	ND	ND
E. coli	Ethanol extract	ND	ND	ND	ND
	Oil extract	ND	ND	ND	ND
C. albicans	Ethanol extract	ND	ND	ND	ND
	Oil extract	10.68 ± 0.49	ND	ND	ND

* Positive controls: inhibition zone of erythromycin (0.015 mg) for *S. aureus* and *S. epidermidis* and were 23.32 ± 0.24 and 27.29 ± 0.65 mm; the inhibition zone of clindamycin (0.002 mg) for *C. acnes* was 46.07 ± 0.50 mm; inhibition zone of ampicillin (0.01 mg) for *E. coli* was 12.62 ± 0.51 mm; inhibition zone of fluconazole (0.025 mg) for *C. albicans* was 24.65 ± 0.25 mm.

Negative control was 95% ethanol; ND = not determined

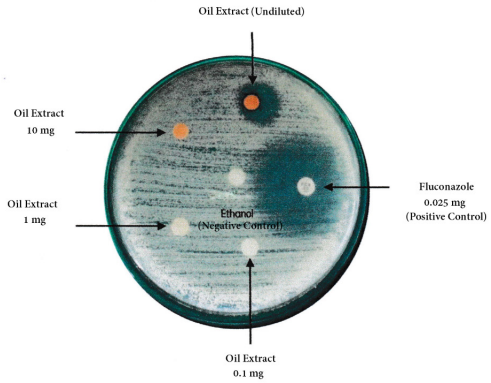

Figure 1: Inhibition zone of undiluted oil extract (100 mg) compared to fluconazole (0.025 mg)

RESULTS AND DISCUSSION

Each extract was viscously crimson in color with a distinct odor, and the extraction yield was up to 10%. Table 1 shows capsicum extracts' bioactive components, including capsaicin and TPC. The TPC and capsaicin in the ethanol extract were 2.50 ± 0.13 mg GAE/g and 0.0104 ± 0.0 mg/100 mL, while in the oil extract were 0.0020 ± 0.0 mg/100 mL and 1.05 ± 0.05 mg GAE/g. Because of the minimal amount of capsaicin in the extract, it was determined that "Bang Chang chili pepper" is non-pungent capsicum (0-700 SHU). Antimicrobial of this chili pepper was found in only oil extract that was inhibited against to *C. albicans* (Figure 1). Whereas, both extracts could not inhibit other pathogenic bacteria (Table 2). Both extracts (0.0001–1.0 mg/mL) had no effect on human fibroblast cells, implying that they are non-toxic.

The finding may imply that non-pungent capsicum strains cannot inhibit bacterial growth due to the low amount of phenolics and capsaicin. The temperature of capsicum extraction is one of factor that varying degrees of inhibition from pungent compounds in capsicum species (capsaicin and dihydrocapsaicin) against bacteria including *Bacillus cereus, B. subtilis, Clostridium sporogenes, C. tetani, and S. pyogenes*.[31] Since, capsaicin possesses antimicrobial properties, which are potent natural inhibitor against pathogenic microorganisms in food.[32] However, they depended on various factors, such as capsicum variety and temperature of extraction as our finding. Furthermore, caffeine, quercetin, and kaempferol are powerful antimicrobial phenolic compounds in capsicum, and several types of interaction, such as synergism, additive, and indifferent, have been reported.[33] Hence, medicinal use of capsaicin or capsicum extract is need to adjust on appropriate formulation and evaluation of their products are necessary to evaluate antibacterial or antifungal properties.[34]

CONCLUSION

Capsaicin, phenolic compounds, and antimicrobial activity of ethanol and oil extracts of the Thai cultivar chili pepper "Bang Chang" (*Capsicum annuum* var. *acuminatum*) were investigated. The ethanol extract contained more capsaicin and phenolic components than the oil extract. Both of extracts were non-toxic with human skin fibroblast. As a result, oil extract

was favored for *C. albicans* suppression. This pepper extract can be used as an antifungal agent, and a pharmaceutical formulation must be developed.

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