RESEARCH ARTICLE

In-vitro Antioxidant and Anti-Inflammation Activities of Ethanol Extract from "Bang Chang" Thai Cultivar Chili Pepper (*Capsicum annuum Var. acuminatum*)

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ABSTRACT

Chili peppers (*Capsicum* spp.) are really important crops in several Asian countries. In Thailand, chili pepper cultivars are also a wide range of flavors, colors, shapes, and spiciness levels. The cultural importance in Thai cuisine. The "Bang Chang chili pepper" (*Capsicum annuum* var. *acuminatum*), a Thai cultivar of capsicum, had first cultivated in Bang Chang subdistrict, Samut Songkhram, Thailand. This study aimed to determine capsaicin, phenolic compounds and flavonoids and screen ethanol extract's biological activities. The extract's average capsaicin and total phenolic content (TPC) was 10.4 ± 0.3 (g/100 mL and 2.50 ± 0.13 mg GAE/g, while it could not determine flavonoids. The low amount of capsaicin in extract was defined this cultivars as non-pungent capsicum (0-700 Scoville Heat Units, SHU). This extract was strongly scavenged NO and DPPH radicals (IC_{50} = 0.09 ± 0.02 and 14.88 ± 1.59 mg/mL). There was also exerted *in-vitro* anti-inflammation activity by suppression of albumin breakdown (IC_{50} = 0.51 ± 0.05 mg/mL), which, can comparable to control, diclofenac diethyl ammonium (IC_{50} = 0.45 ± 0.01 mg/mL). Therefore, lipid peroxidation was weakly inhibited (IC_{50} >1,000 mg/mL) and unable to trap metals compared to vitamin E and EDTA. According to our finding, this extract was exerted biological properties like antioxidant and anti-inflammation, as well as being non-pungent, it can be used externally for medicinal purposes like as a muscle relaxant and massage oil to reduce subcutaneous fat. This extract can be used as a condiment in Asian recipes and other healthful dishes.

Keywords: *Capsicum annuum var. acuminatum,* Chili pepper, Antioxidant, Anti-inflammation.

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INTRODUCTION

Chili peppers (*Capsicum* spp.) are really important crops in several Asian countries such as China, India, Indonesia, Thailand, Vietnam, and Bangladesh. There are agricultural and economic aspects, which are play crucial role as the important spice of cuisines in Asian food cultures.[1,2] In Thailand, cultivation areas vary in climates and geographical conditions, ranging from mountains, river plains and coastal areas in northern, central and southern regions. Due to different growth conditions, chili pepper cultivars also have a wide range of flavors, colors, shapes, and spiciness levels. The cultural importance in Thai cuisine on the difference of chili peppers and other spices on popular Thai dishes i.e., "Tom Yum Goong", spicy shrimp soup and "Som Tum", green papaya salad. Chili peppers are a significant export to various Asian countries with high culinary demand. There are also processed as many preserved products i.e., sauces, pastes, and dried forms. Hence, the invention of value-added products from chili peppers and its by-products are realized in Thailand by an increment of country income.[3-8]

Capsaicin and capsaicinoids contained in chili peppers are unique bioactive compounds and are responsible for its heat sensation. There are activated heat receptors of sensory nerve in the mouth and skin, leading to the perception of spiciness. The spiciness of chili pepper is calculated from their concentrations and explained as Scoville Heat Units (SHU).[7-9] Capsaicin contributes health benefits, which could possess antioxidant and anti-inflammatory properties. Their antioxidant can protect cellular damage from oxidative stress, potentially reducing the risk of chronic diseases including cardiovascular and neurodegenerative diseases. Capsaicin may act as an antioxidant by scavenging reactive oxygen species (ROS) and upregulating antioxidant enzymes, such as superoxide dismutase (SOD) and catalase. In addition, capsaicin may indirectly contribute antioxidant by reducing chronic inflammation that increases oxidative damage.[7-14] *Capsicum annuum* var. *annuum*, commonly known as "bell pepper", offers various health benefits due to its nutritional content. There is also high antioxidant content rather than

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capsaicin and capsaicinoids i.e., carotenoids and vitamin C.[15] Capsicum peppers, including *C. annuum* var. *annuum*, contain various phenolic compounds and flavonoids such as, capsanthin, quercetin, luteolin and rutin. They contribute color and flavor of pepper and provide potential health benefits, such as antioxidants, anti-hypertension, and anti-inflammation.[16, 17] The "Bang Chang chili pepper" (*C. annuum* var. acuminatum), a Thai cultivar of capsicum, had first cultivated in Bang Chang Subdistrict, Samut Songkhram, Thailand. The nutrient content and antioxidant activity had already been disclosed.[18, 19] This study aimed to determine capsaicin, phenolic compounds and flavonoids, and screening on the biological activities of ethanol extract from sundried "Bang Chang chili pepper", such as antioxidant and anti-inflammation, were assessed *in-vitro*. The discovery can be applied in the formulation and manufacturing of pharmaceutical or nutraceutical goods that contain chili pepper extract.

MATERIALS AND METHODS

Cultivation, Harvesting, and Extraction of Pepper

The Tropical Vegetable Research Center at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand provided the seeds for the "Bang Chang" cultivar chili pepper (*C. annuum* var. *acuminatum*), which were then planted in the Samut Songkhram Campus of Suan Sunandha Rajabhat University, Thailand, as the original area, from November 2022 to February 2023. Prior research[18,19] has validated plant identification. Sun-dried chili peppers were created by harvesting red peppers or ripe fruits. Chili peppers that had been sun-dried were deemed sufficient because their moisture level was less than 1%. Fruits had their pedicels removed, sorted, and powdered the remaining material. In 100 g of chili pepper powder was macerated in 500 mL of pure ethanol to extract the compounds.

Phytochemical Analysis

Capsaicin

Capsicum extract was resolved with 70% v/v acetonitrile in water (mobile phase), adjusted concentration to 10.0 mg/mL and filtered prior to transferring sample vial. Standard capsaicin (Sigma-Aldrich, USA) was similarly prepared as sample and stock solution was 1.0 mg/mL. The chromatographic condition included mobile phase: 70% v/v acetonitrile in water; column: ACE Generix 5 C18 (4.6 × 250 mm, 5 mm); injection volume: 20 µL; flow rate: 1.0 mg/mL; detector: deuterium lamp 280 nm. A photodiode array detector represented the signal as a chromatogram, which corresponded to standard capsaicin. Capsaicin retained for around 5.0 minutes. By comparing the amount of capsaicin in sample to a standard, the amount of capsaicin was determined. Scoville Heat Units (SHU), a measure of pungency, were approximations.[20]

Total phenolic content

Total phenolic content (TPC) was determined by Folin-Ciocalteu assay. Extract was dissolved in dimethylsulfoxide (DMSO) and mixed with Folin-Ciocalteu reagent and a suitable alkaline solution. The resulting blue color was proportional to the total phenolic content and quantified using a spectrophotometer at 760 nm. The measurement was expressed as mg of gallic acid equivalents per g (mg GAE/g) of extract.[21]

Total flavonoid content

The aluminum chloride colorimetric method is widely used on quantifying total flavonoid content (TFC). This method is based on the ability of flavonoids to react with aluminum chloride under acidic conditions to form a complex with a yellow color. The intensity of the color is directly proportional to the concentration of flavonoids in extract. A spectrophotometer-equipped microtiter plate reader monitored the absorbance of the reaction mixture at 515 nm. TFC was measured against the standard curve of quercetin (HWI Analytik GmBH, Germany) and results were calculated and expressed as mg of quercetin equivalent per g (mg QE/g) of the extract.[22]

Antioxidant assays

Extract was diluted by absolute ethanol and each concentration of extract (0.001, 0.01, 0.1, 1.0 and 10 mg/mL) was filled in a microtiter plate. The antioxidant assays were included 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide (NO) radical scavenging assay, ferrous iron-ferrozine complex and ferric iron-thiocyanate complex methods, which were monitored the reduction of DPPH radicals, Griess reagent reaction, metal chelation and lipid peroxidation, respectively.[22-25] Micro-titer plate readers were used to track the absorbance reaction mixture for each assay at its maximum wavelength (max). As results, a 50% inhibitory concentration (IC_{50}) of extract was calculated from triple measurements. Vitamin C and vitamin E were positive controls for DPPH and NO radical scavenging assays and ferrous iron-ferrozine complex, respectively. While ethylenediaminetetraacetic acid (EDTA), the metal chelator, was positive control for ferric iron-thiocyanate complex methods.[23-25]

In-vitro anti-inflammation assay

Evaluation anti-inflammatory property was performed by albumin degradation test. Briefly, capsicum extract was dissolved in 20% Tween 20 for 5 minutes while being centrifuged at 150 rpm. The supernatant concentration was changed to 0.01, 0.1, 1, 10, and 100 mg/mL. At 72°C, albumin solution was incubated with each sample for 5 minutes. A positive control was diclofenac diethyl ammonium, which was utilized to assess albumin absorption. Reduction in albumin degradation was used to illustrate anti-inflammatory action.[26]

Analysis of Statistics

Descriptive statistics were used to represent the bioactive components, antioxidant, and anti-inflammatory properties of ethanol extract from "Bang Chang chili pepper" and compare them to controls.

RESULTS AND DISCUSSION

Ethanol extract was viscously red color with specific odor and the yield of extraction was up to 10%. Capsaicin, TPC were among the bioactive components of the capsicum extract that

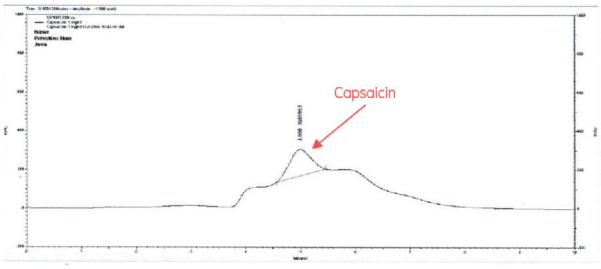
were represented as Table 1. However, there was unbale to detect TFC in this study. As chromatograms, capsaicin retention time from extract corresponded with standard capsaicin (Figure 1). The average of capsaicin and TPC in the extract was 10.4 ± 0.3 µg/100 mL and 2.50 ± 0.13 mg GAE/g, while it was unable to determine flavonoids. The low amount of capsaicin in extract was calculated and defined that "Bang Chang chili pepper" is non-pungent Capsicum (0-700 SHU).[27,28] When compared to vitamin C, this extract was strongly scavenged NO and DPPH radicals (IC_{50} = 0.09 ± 0.02 and 14.88 ± 1.59 mg/mL). There was also exerted in vitro anti-inflammation activity by suppression of albumin breakdown (IC_{50} = 0.51 ± 0.05 mg/mL). This anti-inflammation activity can be comparable to control, diclofenac diethyl ammonium (IC_{50} = 0.45 ± 0.01 mg/mL). Therefore, lipid peroxidation was weakly inhibited (IC_{50} > 1,000 mg/mL) and unable to trap metals compared to vitamin E and EDTA, respectively.

When tested by DPPH, ABTS radical scavenging, and ORAC assays, the "Bang Chang" chili pepper in hexane demonstrated better antioxidant activity than in ethanol and other Cayenne chili peppers. Additionally, the non-polar compartment had higher concentrations of β-carotene and vitamin E, whereas phenolic compounds are commonly contained in polar constituents.[19] Modulating oxidative stress and avoiding chronic illnesses are both important. Thus, applications for capsicum uses include food coloring, spices, muscle relaxants, vasodilators, and cellulite reducers. Because of this, the bioactive antioxidants in peppers fluctuate and are regulated by a number of pre- and post-harvest variables.[29] The extract had anti-inflammatory and antioxidant diabetic properties whereas there was unable to inhibit peroxidation of lipid and metal accelerate oxidation. It may be due to capsaicin and its derivatives being non-polar and yield lower when ethanol extracts. Capsaicin can help people lose weight by activating receptors for transient receptor potential cation channel sub-family V member 1 (TRPV1) channels, which enhance fat metabolism while lowering energy expenditure and thermogenesis. Additionally, there are methods to treat the metabolic syndrome, which includes obesity, diabetes, and cardiovascular disease.[30] Additionally, the capsaicin and quercetin found in chili pepper fruit significantly reduced hyperglycemia in an animal model.[31] According to what we discovered, extraction of "Bang Chang" chili pepper from ethanol, and because it has biological properties like antioxidant and anti-inflammation, as well as being non-pungent, it can be used externally for medicinal purposes like as a muscle relaxant and massage oil to reduce subcutaneous fat. This extract can be used as a condiment in Asian recipes and other healthful dishes.

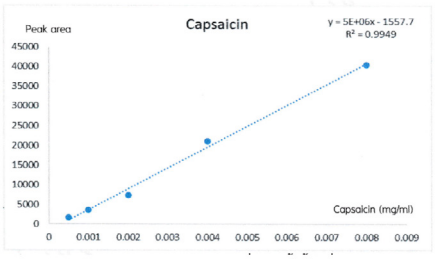
Table 1: Bioactive compounds contained in capsicum extract*

Compound (units)	*Capsaicin (µg/100 g)*	*TPC (mg GAE/g)*	*TFC (mg QE/g)*
Ethanol extract	10.4 ± 0.3	2.50 ± 0.13	ND

*All parameters were calculated from triplicated measurements; GAE = gallic acid equivalent; QE = quercetin equivalent; ND = not determined



(a)



(b)

Figure 1: (a) Peak of capsaicin in chromatogram (RT = 4.095 ± 0.047 min): (b) Standard curve was used to calculated (with peak area) capsaicin concentration contained in Capsicum extract

Table 2: Antioxidant and anti-inflammation activities of chili pepper extract

Assay	*DPPH*	*NO*	*LPI*	*MC*	*ALB*
	(mg/mL)	*(mg/mL)*	*(mg/mL)*	*(mg/mL)*	*(mg/mL)*
Ethanol extract	14.88 ± 1.59	0.09 ± 0.02	>1,000	ND	1.09 ± 0.04
Vitamin C	0.04 ± 0.01	0.02 ± 0.00	-	-	-
Vitamin E			0.04 ± 0.00	-	
EDTA				0.04 ± 0.00	
Diclofenac diethyl ammonium	-	-			0.42 ± 0.0

[a]IC_{50} = 50% of inhibitory concentration calculated from triplicated measurements or more; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; NO = nitric oxide scavenging activity; LPI = inhibition of lipid peroxidation; MC = metal chelating activity ALB = anti-inflammation by albumin degradation inhibition; ND = Not determine

CONCLUSION

The bioactive components and biological activity of the "Bang Chang" Thai cultivar chili pepper (*Capsicum annuum* var. *acuminatum*) were examined after it had been extracted using ethanol. The degree of capsaicin was mild and unpungent. Antioxidant and anti-inflammatory properties were present in the extract. This pepper extraction can be used as a food flavoring and for external medical purposes.

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