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"Bang Chang", Thai Cultivar Chili Pepper (*Capsicum annuum* var. *acuminatum*) Extract in Rice Bran Oil and its Biological Activities

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ABSTRACT

The Thai cultivar Capsicum, namely "Bang Chang chili pepper" (*C. annuum* var. *acuminatum*), is originally planted in Samut Songkhram, Thailand. Rice bran oil (RBO) is a healthy oil for preventing cardiovascular diseases. The study aimed to extract this chili pepper using RBO maceration, which was deemed safer. Capsicum extract was determined for capsaicin and β-carotene contents as bioactives of Capsicum and γ-oryzanol and α-tocopherol as bioactives of RBO. Total phenolic content (TPC) was also monitored. The extract was further evaluated for *in vitro* biological activities, including antioxidant, anti-inflammatory, anti-obesity and anti-diabetic properties. Capsaicin contained in the extract was 0.002±0.0 mg/100 g non-pungent (0-700 SHU). Therefore, β-carotene was 3,489.5±15.2 µg/100 g, higher than dried chili pepper. γ-oryzanol and α-tocopherol contained in the extract were 4,939.6±11.0 mg/kg and 20,090±45.3 µg/100 g, which may affect its biological activities. However, TPC was a low amount (1.05±0.05 mg GAE/g). Capsicum extract moderately scavenged DPPH radicals (IC_{50} = 72.46±8.8 mg/mL) and preferred anti-inflammatory activity by inhibiting albumin degradation (IC_{50} = 1.09±0.04 mg/mL) and inhibiting NO production from macrophage cells (21.31±4.26%, at 0.1 mg/mL). Capsicum extract exerted in vitro anti-diabetic activity by inhibiting α-amylase and α-glucosidase (IC_{50} = 1.35±0.26 and >1,000 mg/mL), while there was a lack of anti-lipase. Capsicum extract (0.0001-1.0 mg/mL) was non-toxic against human fibroblast cells. In conclusion, the simple extraction of this Capsicum within RBO contained bioactive compounds with non-pungency and exhibited biological properties for health benefits with non-toxicity that can be applied for external medicinal use and food seasoning.

Key words: Antidiabetics, Anti-inflammation, *Capsicum annuum* var. *acuminatum*, Chili pepper, Rice bran oil.

INTRODUCTION

Capsicum annuum, commonly known as chili or pepper, belongs to the Solanaceae family. The fruits of chili are widely used as food spices and herbal ingredients (Kobata *et al*., 1998). They vary in sizes, colors, shapes and levels of pungency (FAO, 2001). Asia is a large area for pepper and chili production, especially in China and India (FAO, 2019; OECD, 2006). Capsaicin and its derivatives, capsaicinoids in Capsicum, are alkaloids that provide the pungency property. Carotenoids are the primary pigments in Capsicum that give its fruits color (AK, 2003). Other phytochemicals contained in Capsicum include volatiles, fatty acids, phenolics, vitamins and minerals (OECD, 2006). Capsaicinoids exhibit antioxidant properties and reduce the risk of cardiovascular diseases by inhibiting lipid peroxidation, especially in low-density lipoproteins. Additionally, capsaicin in fats and oils prevents the thermal oxidation of pepper and canola oils during frying and they are similar to tocopherol (Ahuja *et al*., 2006; Yang *et al*., 2010; Si *et al*., 2012). The hydroxyl moiety of capsaicinoids is the functional group responsible for antioxidant properties by preventing the oxidation of polyunsaturated fatty acids (Si *et al*., 2012). Carotenoids are commonly found in fruits and vegetables, reducing the occurrence of chronic diseases due to their antioxidants (Da Silva Antonio *et al*., 2018; Kaur and Kapoor, 2001). Capsicum fruits are rich in carotenoids and promote human health benefits. Sun-dried red peppers have higher carotenoid content and bioaccessibility than orange or yellow peppers under the same conditions (Krinsky, 2001; Pugliese *et al*., 2013). In Thailand, three species of the Capsicum genus are essential ingredients in Thai food, including *C. annuum* L., *C. chinensis* Jacq. and *C. frutescens* L., with various cultivars (Smitinand, 2001). The capsaicin contents of Thai *C. frutescens* and *C. annuum* fruits are 0.47-0.79% and 0.00-0.53% per

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dry weight, respectively; while the carotenoid contents are 0.23-0.48% and 0.06-0.55% per dry weight, respectively. Capsaicin and carotenoids are essential active constituents in Thai Capsicum varieties, useful for evaluating their cultivars for marketing or demand purposes (Boonyamanop, 1993; Kadwey *et al*., 2015). The Thai cultivar Capsicum, namely "Bang Chang chili pepper" (*C. annuum* var. *acuminatum*), is originally planted in Bang Chang subdistrict, Samut Songkhram, Thailand. Its nutritional value and antioxidant activity have been reported (Kaewdoungdee and Tanee, 2013; Sudjaroen, 2014). Rice bran oil (RBO) is a healthy oil favored for Asian food cooking. The World Health Organization (WHO) and the American Heart Association (AHA) have recommended RBO intake for preventing cardiovascular diseases due to its appropriate ratio of fatty acids and phytochemicals, such as γ-oryzanol, tocopherols, squalene and phytosterols (Lai *et al*., 2019; Roger *et al*., 1993; Sugihara *et al*., 2010; Zhang *et al*., 2010). The conventional solvent extraction of capsaicinoids from Capsicum is commonly applied, with methods dependent on various conditions such as the type and amount of solvent, ratio of solvents, duration, polarity and extraction steps (Fabela-Morón, 2019; Castro-Muñoz, 2022). Therefore, the application and safety of chemical solvents during extraction need to be considered, with awareness of their utilization in healthcare products. Hence, we were interested in capsaicinoid extraction from sun-dried Bang Chang chili pepper by maceration in RBO, which was safer for medicinal and nutraceutical uses compared to chemical solvents. Capsicum extract in RBO was determined for capsaicin and β-carotene contents as bioactive constituents from Capsicum, while γ-oryzanol and α-tocopherol were bioactive constituents from RBO. The extract was further evaluated for in vitro biological activities, including antioxidant, anti-inflammation, anti-obesity (anti-lipase) and anti-diabetic properties. The findings can be applied to Capsicum or chili pepper extract in natural oils such as RBO for the formulation and preparation of healthcare products.

MATERIALS AND METHODS

Pepper cultivation, harvesting and extraction

The seeds of the "Bang Chang" cultivar chili pepper (*C. annuum* var. *acuminatum*) were obtained from The Tropical Vegetable Research Center, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand and planted in Samut Songkhram Campus, Suan Sunandha Rajabhat University, Thailand, as the original area from November 2022 to February 2023. Plant identification was confirmed according to a previous study (Kaewdoungdee and Tanee, 2013; Sudjaroen, 2014). Red peppers or ripe fruits were harvested and preserved as sun-dried chili peppers. Sun-dried chili peppers were qualified by monitoring their moisture content, which was lower than 1%. The pedicels were removed and separated from the fruits and the remaining part was ground to a powder form. Five hundred grams of chili pepper powder was ground and extracted with one liter of RBO by maceration within three days. The Capsicum extract in RBO was filtered to remove waste matter, transferred into an amber glass bottle and kept at 4°C.

Phytochemical analysis

Capsaicin

Capsicum extract and dried chili pepper were prepared with acetone and ethanol according to the AOAC official method. The capsaicin content was determined using an Agilent HPLC system equipped with ZORBAX C18 (4.6 × 250 mm, 5 mm). A sample (10 µl) was injected through methanol/water (70:30 v/v) and the flow rate was 1.0 ml/min for 15 min. The photodiode array detector and mass spectrophotometer represented the signal as a chromatogram and mass spectrum, respectively, which corresponded to standard capsaicin. The retention time of capsaicin was around 8.5 min at UV wavelength (λ = 228 and 280 nm). Capsaicin in each sample was quantified by comparison with standard capsaicin (Sigma, USA) and reported as mg/100 g or mg%. The pungency level in Scoville Heat Units (SHU) was approximated using the capsaicin content in ppm × 15.

β-carotene

A sample (100 mg) was homogenized in 3.0 ml of 95% hexane/ethanol/acetone (2:1:1 v/v) and 5 ml of distilled water was added. The mixture was centrifuged at 5,000 rpm for 10 min. The supernatant (5 ml) was adjusted with 95% hexane (5 ml). β-carotene was determined using an Agilent HPLC system equipped with ZORBAX C18 (4.6 × 250 mm, 5 mm). A sample (20 µl) was injected through hexane/water (95:5 v/v) and the flow rate was 1.0 ml/min for 10 min. The retention time of β-carotene was around 4.0 min at λmax = 470 nm. β-carotene in each sample was quantified by comparison with standard capsaicin (Sigma, USA) and reported as µg/100 g or µg%.

γ-oryzanol and α-tocopherol

γ-oryzanol and α-tocopherol analysis were carried out by the Central Laboratory Co., Ltd., Bangkok, Thailand. γ-oryzanol was detected and quantified by an HPLC system equipped with ZORBAX C18 (4.6 × 250 mm, 5 mm) and a PDA detector at 325 nm. The mobile phase consisted of acetonitrile/methanol/isopropanol (50:45:5 v/v). While the mobile phase of α-tocopherol was methanol/water (94:6) and the fluorescence detector was excited at 290 nm and emitted at 330 nm.

Total phenolic content

The Capsicum extract was measured for total phenolic content (TPC) using the Folin-Ciocalteu method and equipped with a spectrophotometer at 760 nm. TPC was represented as mg of gallic acid equivalent (GAE) per g.

In vitro biological assays

Antioxidant assay

DPPH scavenging activity: DPPH radicals in the presence of the extract (0.01-1 mg/ml) were scavenged and their absorbance was monitored. DPPH radicals (6 $\times 10^{-5}$ M) and ascorbic acid were used as negative and positive controls, respectively.

Nitric oxide scavenging activity

NO radicals in the presence of the extract (0.01-1 mg/ml) were scavenged and the absorbance of the reaction from the Griess reagent was monitored. Ascorbic acid was used as a positive control.

Anti-lipase assay

Capsicum extract (0.001-10 mg/ml) was dissolved in 10% (v/v) DMSO. Orlistat (0.0005-5.0 mg/ml) as a positive control was also adjusted with 10% (v/v) DMSO. Enzymatic inhibitory activity was evaluated by monitoring the absorbance (λmax = 415 nm) of p-nitrophenol, the product from *p*-nitrophenyl butyrate catalyzed by pancreatic lipase.

Anti-α-glucosidase assay

Capsicum extract (0.001-10 mg/ml) was dissolved in 10% (v/v) DMSO, while acarbose (0.0005-5 mg/ml) as a positive control was dissolved in phosphate buffer. Enzymatic inhibitory activity was evaluated by monitoring the absorbance (λmax = 415 nm) of p-nitrophenol, the product from *p*-nitrophenyl-α-D-glucopyranose catalyzed by α-glucosidase.

Anti-α-amylase assay

Capsicum extract and the positive control were similar to the anti-α-glucosidase assay. While the enzymatic inhibitory activity was evaluated by monitoring the absorbance (λmax = 450 nm) of reducing sugar, the product from starch catalyzed by α-amylase.

Cytotoxicity test

The Capsicum extract was dissolved in DMEM, containing DMSO (10%), FBS (10%), penicillin/streptomycin (1%), filtered with a 0.2 µM membrane and adjusted to the concentration within each well of the microtiter plate by sterile culture medium (0.0001, 0.001, 0.01, 0.1 and 1.0 mg/ml). Each sample or control well was suspended in human skin fibroblasts (2.2-3.3 $\times 10^4$ cells/ml) and incubated for 48 h. Cell cytotoxicity was determined by sulforhodamine B staining with viable skin cells. Cell viability of human skin fibroblast cells against the extract was explained as cell viability (%), which was calculated from four-time repeated experiments. The positive control (cytotoxic) was sodium lauryl sulfate and the negative control (non-toxic) was DMEM.

Anti-inflammation assay

Albumin degradation test

The capsicum extract was dissolved with 20% Tween 20 and centrifuged at 150 rpm for 5 min. The supernatant was adjusted to 0.01, 0.1, 1, 10 and 100 mg/ml. Each sample was incubated with albumin solution at 70±2°C for 5 min. The absorbance of albumin was monitored and diclofenac diethyl ammonium was used as a positive control. Anti-inflammatory activity was represented as a reduction of albumin degradation.

Reduction of NO produced from lipopolysaccharide (LPS)-induced macrophage cells

Mouse macrophage cell (RAW264.7) was cultured in Dulbecco's modified Eagle's medium, DMEM (Invitrogen, USA), containing fetal bovine serum, FBS (10%) and penicillin/streptomycin (1%), in a humidified incubator (5% CO_2) at 37°C for 24 h. The cell culture was transferred to a 24-well plate and suspended to 1 $\times 10^5$ cells with 500 µl of medium/well. Each cell culture well was treated with different concentrations of extract (or controls) for 1 h and with LPS for an additional 24 h. After that, the supernatant of the culture medium from each well was determined for NO production. Culture medium (50 µl) and Griess reagent (100 µl) were mixed in a 96-well plate and stood for 10 min at room temperature. The absorbance of the reaction mixture was monitored by a microtiter plate reader. The positive control was triamcinolone acetonide (0.1 mg/ml).

Data analysis

The bioactive compounds of dried chili pepper and Capsicum extract were represented as the mean and standard deviation of concentration. In vitro biological activities of chili pepper extract were also analyzed by descriptive statistics and compared with controls. Data were calculated from triplicate experiments for each parameter.

RESULTS AND DISCUSSION

The appearance of ripe and dried "Bang Chang" chili pepper was 1.9-2.5 cm × 10-15 cm, with shiny vermillion color and flesh thickness was 2.0-3.0 mm. Capsicum extract in RBO was viscous and tangerine in color (Fig 1).

Bioactive compounds of the Capsicum extract, including TPC, capsaicin, β-carotene, γ-oryzanol and α-tocopherol, were monitored (Table 1). Capsaicin and β-carotene were detected as chromatograms in Fig 2 and 3, respectively. The capsaicin level in the extract (0.002±0.0 mg/100 g) was 0.5 times that of dried chili pepper on an extract ratio basis. Based on the capsaicin level, the pungency levels of dried chili pepper and extract were non-pungent (0-700 SHU) (AOAC, 2016; Kraikruan *et al*., 2008; Collins *et al*., 1995. Therefore, the β-carotene in this extract (3,489.5±15.2 µg/100 g) was higher than that in dried chili pepper, which may have been added from RBO.

γ-oryzanol and α-tocopherol of the capsicum extract were also determined from the RBO extractant. Hence, this simple oil extraction still retained capsaicin and β-carotene from chili pepper and γ-oryzanol and α-tocopherol from RBO, which affected its biological activities. Three bioactive compounds of capsicum fruits are mainly

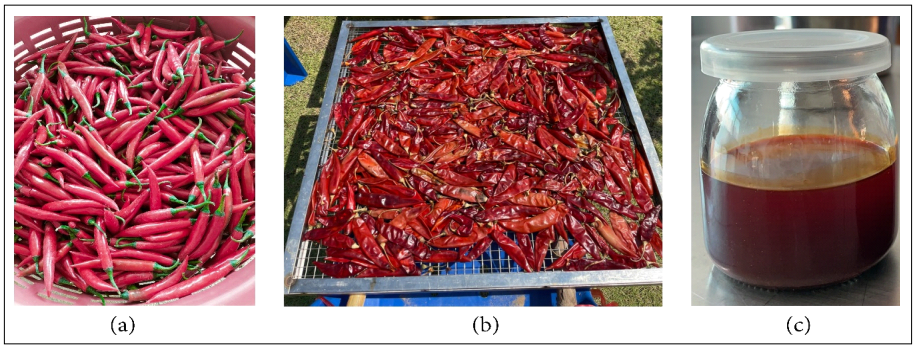


Fig 1: Appearance of ripe, dried "Bang Chang" chili pepper and *Capsicum* extract in RBO.

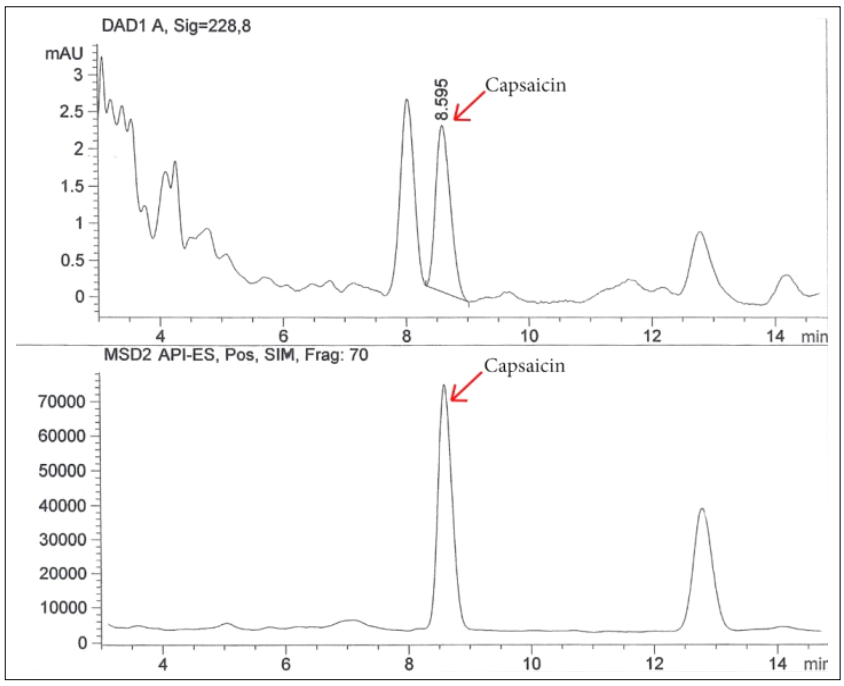


Fig 2: Chromatogram and mass spectra of capsaicin were detected from *Capsicum* extract.

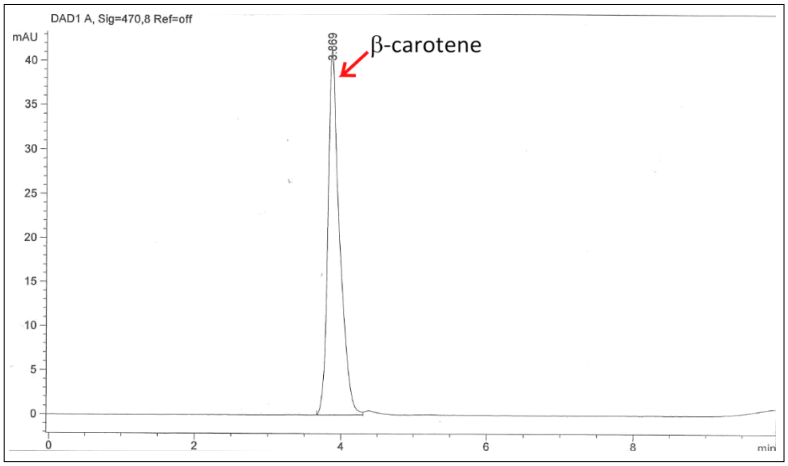


Fig 3: Chromatograms β-carotene was detected from *Capsicum* extract.

carotenoids, flavonoids and capsaicinoids (Collins *et al*., 1995). This chili pepper fruit had low capsaicin with non-pungency, which was similar to C. annuum Cayenne. Additionally, the fruit characteristics of both chili peppers were similar (Bae *et al*., 2014). Our result confirmed that sun-dried red peppers had a higher carotenoid content with bioaccessibility (Pugliese *et al*., 2013) and could protect against lipid peroxidation in oil, as shown in previous studies (Si *et al*., 2012). However, our study only monitored TPC in the extract rather than flavonoids.

This extract moderately scavenged DPPH radicals (IC_{50} = 72.46±8.8 mg/ml) and poorly inhibited NO radicals (IC_{50}>1,000 mg/ml) when compared with ascorbic acid. There was also preferred anti-inflammatory activity by inhibition of albumin degradation (IC_{50} = 1.09±0.04 mg/ml) and inhibition of NO production from macrophage cells (21.31±4.26%, at 0.1 mg/ml). In addition, the extract exhibited anti-diabetic activity by inhibiting α-amylase and α-glucosidase (IC_{50} = 1.35±0.26 and >1,000 mg/ml), while it was unable to inhibit lipase (Table 2 and 3).

The extract (0.0001-1.0 mg/ml) was unaffected against human fibroblast cells, implying that it is non-toxic (Table 4). Moreover, capsaicin and quercetin contained in chili pepper fruit had a synergistic effect in alleviating hyperglycemia in animal models (Mandal *et al*., 2022; Varghese *et al*., 2016). Based on our findings, simple extraction of "Bang Chang" chili pepper within RBO is easy to perform and its biological properties, including antioxidant, anti-inflammation and anti-diabetic properties with non-pungency and non-toxicity, can be applied externally for medicinal use, such as muscle relaxants and massage oils for reducing subcutaneous fat. This extract can also be used as a food seasoning for healthy foods, especially in Asian recipes. A previous study of "Bang Chang" chili pepper in hexane had higher antioxidant activity than in ethanol and other Cayenne chili peppers when measured by DPPH, ABTS radical scavenging and ORAC assays.

There was also a higher content of β-carotene, vitamin E and phenolic compounds in the non-polar compartment (Sudjaroen, 2014). Therefore, bioactive antioxidants in peppers vary and are influenced by several pre- and post-harvest factors, modulating oxidative stress and preventing chronic diseases. Applications of Capsicum are varied and include spices, food colorants, muscle relaxants, vasodilators and cellulite reducers (Mandal *et al*., 2022).

Hence, we prepared Capsicum extract by simple RBO extraction, measured bioactive compounds and confirmed its biological activities. The extract showed antioxidant, anti-inflammation and anti-diabetic properties.

Table 1: Bioactive compounds contained in dried chili pepper and extract[a].

Compound (units)	Capsaicin (mg/100 g)	β-carotene (μg/100 g)	γ-oryzanol (mg/kg)	α-tocopherol (μg/100 g)	TPC (mg GAE/g)
Chili pepper extract	0.002±0.0	3,489.5±15.2	4,939.6±11.0	20,090±45.3	1.05±0.05[b]
Dried chili pepper	0.018±0.0	1,566.8±18.1	-	-	-

[a]All parameters were calculated from triplicated measurements; [b]Total phenolic content (TPC) was mg of gallic acid equivalent (GAE) per g; TPC= Total phenolic content.

Table 2: Antioxidant, anti-inflammation and enzymatic inhibitory activities of chili pepper extract.

Assay (IC_{50})	DPPH (mg/ml)	NO (mg/ml)	ALB (mg/ml)	α-GLU (mg/ml)	α-AM (mg/ml)	LP (mg/ml)
Chili pepper extract	72.46±8.8	>1,000	1.09±0.04	>1,000	1.35±0.26	ND
Ascorbic acid	0.02±0.0	0.04±0.01	-	-	-	-
Diclofenacdiethyl ammonium	-	-	0.42±0.0	-	-	-
Acarbose	-	-	-	0.14±0.02	0.007±0.001	
Orlistat	-	-	-	-	-	3.08±0.98

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; ALB= Anti-inflammation by albumin degradation inhibition; α-GLU= α-glucosidase inhibition; α-AM= α-amylase inhibition; LP= Anti-lipase activity; ND= Not determine.

Table 3: Inhibition of NO production from LPS-induced macrophages from chili pepper extract.

Sample	Inhibition of NO production (%)					
(mg/ml)	0.0001	0.001	0.01	0.1	1.0	10.0
Chili pepper extract	ND	16.84±3.64	19.93±2.60	21.31±4.26	19.24±1.32 [a]	-8.93±1.73[a]
Triamcinolone acetonide	26.11±3.36	28.45±4.99	29.91±3.30	30.78±3.22	33.12±3.62	ND

[a]Sample color was interfered NO measurement; ND= Not determined.

Table 4: Cell viability of human fibroblast cells against chili pepper extract.

Sample	Cell survival rate (%)				
(mg/ml)	0.0001	0.001	0.01	0.1	1.0
Chili pepper extract	96.25±2.79	96.15±1.82	93.39±4.80	92.60±5.48	88.31±4.01
SLS	95.23±2.17	94.79±1.13	87.42±5.67	12.14±2.14	8.17±0.39

SLS= Sodium lauryl sulfate.

In our study, this extract lacked anti-obesity properties by being unable to inhibit lipase. However, capsaicin can facilitate weight loss by inducing Transient Receptor Potential Cation Channel sub-family V member 1 (TRPV1) channels, improving fat metabolism, reducing energy expenditure and inducing thermogenesis. It can also improve insulin action and treat metabolic syndrome, including obesity, diabetes and cardiovascular diseases (Varghese *et al*., 2016; Mi *et al*., 2023).

CONCLUSION

The "Bang Chang" Thai cultivar chili pepper (*Capsicum annuum* var. *acuminatum*) was extracted with rice bran oil (RBO) and bioactive compounds and biological activities were measured. Capsaicin levels were low and non-pungent. Therefore, the β-carotene content in the extract was higher than in dried chili pepper. The extract possessed antioxidant, anti-inflammatory and anti-diabetic properties. This simple extraction of pepper was easy to perform and can be applied for external medicinal use and as a food seasoning.

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Conflict of interest

There are no conflicts of interest in this study.

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