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Research Article

Antioxidant and antibacterial properties in Keang-hleung paste and its ingredients

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Abstract

Keang-hleung or yellow curry is a traditional popular spicy-sour curry consumed in southern Thailand. In general, the ingredients used in the paste are turmeric rhizome, garlic and chili; however, some areas may also add galangal rhizome. Some herbs/spices exhibit free radical scavenging features that inhibit bacterial growth and act as natural preservative agents. This study investigates the total phenolic content and the antioxidant and antibacterial activities of Keang-hleung paste and its ingredients. It was found that the total phenolic content of garlic, galangal, turmeric, dried chili and Keang-hleung paste extracts determined by the Folin–Ciocalteu method were 0.3 ± 0.02, 0.2 ± 0.01, 0.3 ± 0.06, 0.6 ± 0.06 and 0.8 ± 0.02 mg GAE/100 g respectively. The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) activity of garlic, galangal, turmeric, dried chili and the paste extracts were 0.38 ± 0.07, 0.65 ± 0.41, 1.01 ± 0.34, 0.28 ± 0.65 and 1.77 ± 0.38 mg GAE/100 g, while the ferric reducing power were 0.03 ± 0.01, 0.05 ± 0.01, 0.07 ± 0.02, 0.07 ± 0.02 and 0.05 ± 0.01 mg GAE/100 g, respectively. For the antibacterial activity it was found that only garlic and the paste could inhibit *Staphylococcus aureus* and *Bacillus cereus*. The minimal inhibitory concentration (MIC) values of garlic and the paste extract on *S. aureus* and *B. cereus* were 0.407 ± 0.003 and 0.407 ± 0.003, 1.306 ± 0.005 and 1.306 ± 0.005 mg/ml, respectively. The minimal bactericidal concentration (MBC) values of garlic and the paste extract on *S. aureus* and *B. cereus* were 3.259 ± 0.001 and 0.815 ± 0.001, 5.224 ± 0.004 and 2.612 ± 0.004 mg/ml respectively.

Keywords: Keang-hleung paste, yellow curry, antioxidation, antibacterial, pathogenic bacteria, Thailand

Introduction

Keang-hleung or yellow curry is a traditional spicy-sour curry and is popular and is consumed in southern Thailand. It is assumed to be a healthy food due to its low sugar, fat and its spices such as turmeric rhizome, garlic and chili. In some areas galangal rhizome may also be added as a preference. Some herbs/spices are classified as natural preservative agents because their active compounds provide free radical scavenging and inhibit bacterial growth. Curcumin, a naturally occurring phytochemical responsible for the colour of turmeric (*Curcuma longa*), shows a wide range of pharmacological properties including antioxidant, anti-inflammatory and anti-cancer effects [1]. Allicin, the principle antimicrobial compound of garlic *(Allium sativum*), is only generated after the cloves are injured and the enzyme alliinase reacts with its substrate alliin, [2]. This compound was found to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria. These included multidrug-resistant enterotoxicogenic strains of *Escherichia coli*, antifungal activity, particularly *Candida albicans*. In addition it showed antiparasitic activity including some major human intestinal protozoan parasites, such as *Entamoeba histolytica* and *Giardial amblia*, and antiviral activity [3]. In Thailand, galangal (*Alpinia galangal*) is used for medicinal purposes, such as for carminative, stomachic, antispasmodic, antichloristic and antibacterial drugs [4], and as an antioxidation [4, 5, 6, 7, 8, 9]. Capsaicin is a compound in chili that could inhibit the growth of *Helicobacter pylori* [10]. Many researchers study individual herbs/spices as food additives, but no scientific data has been reported about combinations of herbs/spices such as Keang-hleung paste as natural preservatives and functional food. The objective of this study was to determine the antimicrobial and antioxidant activities of Keang-hleung paste and its ingredients.

Materials and Methods

Materials and reagents

Turmeric rhizomes (*Curcuma longa*), garlic (*Allium sativum*), chili (*Capsicum frutescense*) and galangal rhizomes (*Alpinia galanga*) were purchased from a local market in Haad Yai, Thailand. All chemicals, reagents, and media were of analytical grade and obtained from Sigma Chemical Co. (St. Louis, MQ, USA) and Merck (Darmstadt, Germany).

Keang-hleung paste and its ingredients

For the preparation of Keang-hleung paste, all spices were sorted, trimmed and washed thoroughly to remove dust and dirt, then weighed according to the recipe. They were then ground with a blender (Moulinex, TYPE 276, France) to make a fine paste. While the individual ingredients were prepared as the paste, 100-g (wet weight) of each sample was extracted with 300 ml of distilled water twice. This was then mixed together before being subjected to filtering through a filter paper (ϕ125 mm, Cat. no. 1001 125, (Whatman Schleicher and Schuell, England) and kept at 4^0C until used.

Total phenolic contents

The total phenolic contents of each sample were determined using a Folin–Ciocalteu assay with slight modification [11]. The reaction mixture contained 1 ml of extract, 0.5 ml of the Folin–Ciocalteu reagent, 1 ml of 10 g/100 ml sodium carbonate and 7.5 ml of distilled water. After 45 minutes of reaction at ambient temperature, the absorbance at 765 nm was measured using a UV-visible spectrophotometer (Jasco V-530, Japan Servo Co., Ltd.). A calibration curve was calculated using standard gallic acid (0.016, 0.008, 0.004, 0.002 and 0.001 mg/ml, r^2= 0.995). The results were expressed on a dry weight basis (dw) as mg gallic acid equivalents (GAE), per 100 g of dry sample.

Antioxidant activity evaluation

Free radical scavenging (DPPH•) assay

Free radical scavenging was determined using the free radical generator DPPH• (2,2-diphenyl-1- picrylhydrazyl) assay based on slight modifications [12]. An aliquot (1 ml) of the serially diluted extract samples was mixed with 1 ml of 500 μM DPPH• solution was added. The mixture was thoroughly mixed using a vortex and kept in the dark for 30 minutes. The absorbance, using a spectrophotometer, was measured later at 518 nm against a blank of ethanol without DPPH•. The results were expressed on a dry weight basis (dw) as mg GAE/100 g dry sample.

Ferric reducing/antioxidant power (FRAP) assay

A FRAP assay was performed using a modified method [13]. Briefly a 150 μl aliquot of properly diluted extract was mixed with 2850 μl FRAP reagent and incubated at 37°C for 4 minutes. The absorbance was then determined at 593 nm against a blank that was prepared using distilled water. FRAP was freshly prepared by mixing 2.5 ml of a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with 2.5 ml of 20 mM $FeCl_3$ $6H_2O$ and 25 ml of 0.3 M acetate buffer at a pH of 3.6. A calibration curve was prepared, using different concentrations of gallic acid (0.016, 0.008, 0.004, 0.002 and 0.001 mg/ml, $r^2 = 0.997$). FRAP values were expressed on a dry weight basis (dw) as mg GAE/100 g dry sample.

Preparation of microorganisms

Staphylococcus aureus and *Bacillus cereus* (ATCC 1778) were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. Each organism was maintained in Nutrient Agar (NA) at 5^{0}C. Stock culture from both organisms was transferred to 5 ml of Brain heart infusion broth (BHI) and incubated at 37^{0}C for 15 hours (cell in log phase). This culture was used for the antimicrobial assay.

Antimicrobial activity evaluation

Minimum inhibitory concentration (MIC)

One loopful of *S. aureus* and *B. cereus* was individually cultured into 5 ml of BHI and incubated in an incubator at 37^{0}C for 15 hours. Each bacterium was diluted with 0.85% sterile sodium chloride to achieve a final concentration of approximately 10^6 cfu/ml. One-ml of the bacterial suspension at 10^6 cfu/ml was transferred into 1 ml of Muller Hinton Broth (MHB) then 1 ml of a series of two-fold dilutions of each extract was added. The MIC of the extracts was regarded as the lowest concentration of extracts that did not permit any turbidity of the tested microorganism [14, 15].

Minimum bactericidal concentration (MBC)

All the tubes were used in the MIC studies that did not show any turbidity in the bacteria and the last tubes with turbidity were determined for MBC. An aliquot of the suspension (0.1 ml) was spread onto Muller Hinton Agar (MHA) and incubated at 35^{0}C for 15 hours. The MBC was the lowest concentration which the initial inoculums were killed as 99.9% or more [14, 15].

Statistical Analysis

Data were subjected to analysis of variance, and mean comparisons were made using Duncan's new multiple range test. Statistical analyses were carried out using the SPSS statistical software version 11 (SPSS, Inc., Chicago, IL).

Results and Discussion

Total phenolic contents and antioxidant of Keang-hleung paste and its ingredients

Table 1 shows the amount of the total phenolic contents of Keang-hleung paste and its ingredients. The highest level of phenolic content of any individual spices/herbs was found in chili, while the lowest content was found in galangal. Surprisingly, when the ingredients were made into Keang-hleung paste, the total phenolic content increased and was higher when described later.

Antioxidant activities

DPPH$^{\cdot}$ free radical scavenging

DPPH is a free radical compound that has been widely used to determine the free radical scavenging capacity of various samples [16, 17] because of its stability (in radical form), simplicity and fast assay [18]. The DPPH free radical scavenging activity of Keang-hleung paste and its ingredients are presented in Table 1. The results showed that the second highest free radical scavenging activity was in turmeric rhizomes that did not show agreement with the total phenolic content. This was probably due to the high activity of the active compound and/or the synergistic effect of curcuminoid compounds, such as curcumin, demethoxycurcumin and bisdemethoxycurcumin. The active ingredients in turmeric rhizomes are a group of phenolic compounds, including curcumin, which is well known for its strong antioxidant activity [19]. However, Jayaprakasha *et al*. [8] reported that the other two curcuminoids were also effective antioxidants. Ruby *et al*. [5] noted that curcuminoid compounds found in turmeric rhizomes were potent scavengers of hydroxyl radicals. Darrick *et al*. [20] also reported that bisdemethoxycurcumin and demethoxycurcumin were good in trapping the DPPH radical. While the lowest free radical scavenging activities were found in garlic and chili, it was found that Keang-hleung paste possessed the highest DPPH free radical-scavenging activity. This may be due to the synergistic antioxidant activity. This is similar to the findings of Shobana and Naidu [21]. They reported that a spice mix (ginger, onion and garlic; onion and ginger; ginger and garlic) showed accumulative inhibition of lipid peroxidation. They exhibited a synergistic property when compared with individual ones.

Ferric reducing/antioxidant power (FRAP)

The highest value of FRAP was found in turmeric and chili (Table1), while the lowest value was in garlic. Making Keang-hleung paste did not improve the FRAP activity compared with turmeric and chili. The antioxidant activities of phenolic compounds are mainly of redox properties, including free radical scavenging, hydrogen donating and singlet oxygen quenching [4]. Pulido *et al*. [22] reported on the reducing capacity of polyphenols, as determined by the FRAP assay. This seems to depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds. It implies that mixing of herbs/spices may or may not improve some antioxidant properties due to chemical reaction.

Table 1. Total phenolic contents and antioxidation properties of Keang-hleung paste and its ingredients.

Sample	Total phenolic contents (mg GAE/100g)	Scavenging radical (mg GAE/100g)	%FRAP (mg GAE/100g)
Garlic	$0.30^{a} \pm 0.02$	$0.38^{a} \pm 0.07$	$0.03^{a} \pm 0.01$
Galangal	$0.20^{b} \pm 0.01$	$0.65^{b} \pm 0.41$	$0.05^{b} \pm 0.01$
Turmeric	$0.30^{a} \pm 0.06$	$1.01^{c} \pm 0.34$	$0.07^{c} \pm 0.02$
Chili	$0.60^{c} \pm 0.06$	$0.28^{a} \pm 0.65$	$0.07^{c} \pm 0.02$
Keang-hleung paste	$0.80^{d} \pm 0.02$	$1.77^{d} \pm 0.38$	$0.05^{b} \pm 0.01$

Each value is expressed as a mean ± SD (n=3)
a-e means that with different letters within a column are significantly different ($p<0.05$).

Antimicrobial activities

The bacterial loads of the different samples are presented in Table 2. The results show that dry chili was heavily contaminated with bacteria, while garlic had low bacterial loads. Spices and natural agricultural seasoning materials are commonly contaminated with microorganisms including bacteria, mould and yeasts [23]. However, the number and type of microorganisms may vary with harvesting, storage, transport and packaging [24]. But when the ingredients were made as Keang-hleung paste, the bacterial population was reduced to around a 2 log cycle when compared with dried chili. This is due to the functioning of allicin in garlic, which is similar to the findings of Siripongvutikorn *et al* [24]. Additionally, the results showed that only garlic could provide minimum inhibitory inhibition (MIC) on both *S. sureus* and *B. cereus.* When a higher concentration of garlic extract was applied the killing activity (MBC) occurred as shown in Table 3. The antimicrobial effect of garlic apparently results from the interaction of sulphur compounds, like allicin, with sulphur (thiol) groups of microbial enzymes such as trypsin and other proteases. This leads to an inhibition of microbial growth [25, 26]. Some researchers have found that capsaicin, the main active compound for pungency or heat sensation, has an antimicrobial property against *Helicobacter pylori* [10]. However, there was no antimicrobial effect in dry chili in this study. This may be because the capsaicin compound has less water solubility [27], and some researchers have also found that ethanolic extract of galangal and turmeric have an antimicrobial capacity [28,29]. This implies that most compounds which have an antimicrobial capacity in galangal and turmeric cannot be dissolved in water or the polar phase condition. It is also possible that the concentration and/or purity of active compounds are not high enough to inhibit the test bacteria, and/or the different test organisms [24]. When the ingredients were blended to become Keang-hleung paste, they showed even less antimicrobial effects than garlic. This suggests that there was a certain amount of allicin derived from the garlic.

Table 2. Bacterial load in Keang-hleung paste and its ingredients.

Samples	Bacteria count (cfu/ml)
Garlic	10^{2}-10^{3}
Galangal	10^{4}-10^{5}
Turmeric	10^{4}-10^{5}
Dry chili	10^{6}-10^{7}
Keang-hleung paste	10^{4}-10^{5}

Each value is expressed as a mean ± SD (n=3)

Table 3. Antimicrobial activities of Keang-hleung paste and its ingredients.

Spices	MIC (mg/ml)		MBC (mg/ml)	
	S. aureus	*B. cereus*	*S. aureus*	*B. cereus*
Garlic	0.407 ± 0.003	0.409 ± 0.003	3.259 ± 0.001	0.815 ± 0.001
Turmeric	0	0	0	0
Galangal	0	0	0	0
Dry chilli	0	0	0	0
Keang-hleung paste	1.306 ± 0.005	1.307± 0.005	5.224 ± 0.004	2.612 ± 0.004

Each value is expressed as a mean ± SD (n=3)

Conclusions

Making Keang-hleung paste could improve the total phenolic content, free radical scavenging and ferric reducing antioxidant powers compared with its individual ingredients. Fresh garlic plays the key role for inhibiting both *S. aureus* and *B. cereus*, whereas dry chili, turmeric rhizome and galangal rhizome had no effect. However, after each ingredient was blended into Keang-hleung paste, further antibacterial properties were shown. In summary, Keang-hleung paste has potential as both an antioxidant and antimicrobial and could be a functional food.

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