การสำรวจการต้านอนุมูลอิสระของน้ำพริกลาบในจังหวัดเชียงใหม่ ลำพูน และลำปาง

Survey of the Antioxidant Activity of Nam Prilk Larb Sold in the Chiang Mai, Lamphun and Lampang

อานง ใจแน่น[1*]

Anong Jainan[1*]

บทคัดย่อ

งานวิจัยนี้เป็นการสำรวจฤทธิ์การต้านอนุมูลอิสระของพริกลาบในจังหวัดเชียงใหม่ ลำพูน และลำปาง โดยใช้วิธีการสุ่มตัวอย่างตามความสะดวกมีจำนวน10 ตัวอย่าง ซึ่งน้ำพริกลาบแต่ละตัวอย่างจำนวน 100 กรัม มาวิเคราะห์หาสารต้านอนุมูลอิสระโดยวิธี DPPH, ABTS และ FRAP ตรวจสอบปริมาณสารประกอบฟีนอลิกและสารประกอบฟลาโวนอยท์ทั้งหมดพบว่า ตัวอย่างน้ำพริกลาบลำดับที่ 10 จากจังหวัดเชียงใหม่มีปริมาณฟีนอลและฟลาโวนอยด์สูงสุด (324.11 mg GAE / 100 g DW และ 253.31 mg CAE / 100 g DW) ได้แก่ DPPH (745.59 mg ascorbic acid / 100 g DW) พบว่ามีค่า ABTS และ FRAP สูงสุดตามลำดับจากสารสกัดหยาบจากตัวอย่างน้ำพริกลาบลำพูนลำดับที่ 6 ซึ่งมีปริมาณแอสคอร์บิกเป็น 465.04 มิลลิกรัม / 100 กรัมและ 29.51 ไมโครลิตร Fe^{2+} / g DW ตามลำดับ สารสกัดจากน้ำพริกลาบแต่ละชนิดมีแนวโน้มที่จะมีสารฟีนอลและฟลาโวนอยด์สูงรวมกับสารต้านอนุมูลอิสระดังนั้นจึงเป็นส่วนผสมในอาหารไทยที่มีประโยชน์ในรสชาติหรือกลิ่นหอมของเครื่องเทศเพื่อสุขภาพ

คำสำคัญ: สารต้านอนุมูลอิสระ น้ำพริกลาบ สารประกอบฟีนอลลิกทั้งหมด สารประกอบฟลาโวนอยด์

Abstract

This research was conducted to survey the antioxidant activity and total phenolic content of Nam Prilk Larb from Chiang Mai, Lamphun and Lampang. Ten convenience samplings with 100 grams of Nam Prilk Larb each was analyzed for antioxidant activities. Ten Larb seasonings from different area in Chiang Mai, Lamphun and Lampang of Thailand were evaluated for total phenolic and flavonoid content including the antioxidant activity (DPPH, ABTS and FRAP). The 10th Larb seasoning from Chiang Mai had the highest total phenolic and flavonoid content (324.11 mg GAE/100 g DW and 253.31 mg CAE/100 g DW), including DPPH activity (745.59 mg ascorbic acid/100 g DW). The highest ABTS and FRAP activity was obtained from the crude extract of 6th Larb seasoning from Lamphun, which was 465.04 mg ascorbic acid/100 g DW and 29.51 µmol Fe^{2+}/g DW, respectively. All of crude extracts from each Larb seasoning tend to have high content of the total phenolic and flavonoid, incorporate with the antioxidant activity. Thus Larb seasoning is a kind of ingredient in Thai food which contains in the benefits of flavors or aroma culinary spices for health.

Keywords: Antioxidant, Nam Prilk Larb, Phenolic Compound, Flavonoid Compound

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[1] อ., โรงเรียนการเรือน สาขาเทคโนโลยีการประกอบอาหารและการบริการ มหาวิทยาลัยสวนดุสิตศูนย์การศึกษานอกที่ตั้งลำปาง 52100

[1] Lecturer, School of Culinary Arts, Program in Culinary Technology and Service, Suan Dusit University, Lampang Center, 52000

[*] Corresponding author: Tel.: 062-1946-293. E-mail address: noi.boy@hotmail.com

Introduction

Thai food is presently appealing to be interest due to the health benefits from the flavors and aroma of culinary spices. As the northern Thai food are commonly made from vegetables, herbs and spices, which are the sources of phytochemicals possess effect of human health. Spicy mincemeat or Larb in Thai, is an identity food in northern Thailand. The main ingredient or seasoning in it is Larb seasoning (Num Prilk Larb). Larb seasoning components include chilli, garlic, shallot, galangal, lemongrass, makwaen, long peper, coriander seed and shrimp paste, almost 80% of whole seasoning. The 20% remaining of Larb seasoning are spices such as cumin, cinnamon, clove, star anise, cardamom, nutmeg, mace, fennel, black pepper, sand ginger, and so on. Owing to Larb seasoning's major ingredients have various bioactivities such as antioxidant, antibacterial, antitumor, antimutagenicity, antidiabetic and anticancer etc [1-2].

The relationship between antioxidant food and health has been widely examined. Numerous diseases such as aging, coronary heart disease, cancer, alzheimer and so on came from the oxidative and free radical reactions in body [3]. There are many researches that report phytochemicals in culinary vegetables and spices have accepted to be the great sources of antioxidant, to prevent diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen and various radicals as damaging side effect of aerobic metabolism. These radicals are possiblythe reason of disorders including cardiovascular malfunctions, tissue injury, DNA damage and tumor promotion [4]. Antioxidants found in food are heterogeneous category of molecules that can safely interact with free radicals and terminate the reaction before vital molecules are dramaged [5].

Culinary herbs and spices have been used for thousands years in medicine and also as preservative agents to extend the shelf-life of food and enhance or improve the flavor and organoleptic properties of different types of food due to their sensory properties [6-7]. The bioactive compounds of these plant-based products have been evaluated, including vitamins, flavonoids, terpenoids, carotenoids, phenolic compounds and minerals [7]. Moreover, there are many researches report that phytochemicals that are beneficial for human, especially polyphenols, such as antioxidant activity, digestive stimulant action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic, anticarcinogenic potential etc [8]. Additionally, polyphenols can extend the shelf-life of rich lipid foods [9].

Therefore, Larb seasoning (Num Prilk Larb) consists of many spices and herbs may possessss antioxidant activity and medicinal properties. The different source of Larb seasoning was collected and investigated the total phenolic and flavonoid contents, including antioxidant activity of each sample.

The antioxidant activities in Larb seasoning or Nam Prilk Laab from different areas in the northern of Thailand were compared by three different methods such as DPPH radical scavenging assay, ABTS radical cation decolorization assay and Ferric reducing antioxidant power assay. Moreover, the total phenolic and total flavonoid contents of each sample were also determined and related to their antioxidant activity.

Materials and Methodology

Materials

(2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) ,(Diammonium Salt) ABTS ,(2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin and ascorbic acid were purchased from Sigma-Aldrich Co. (St Louis, MO). 2,4,6-Tripyridyl-s-triazine (TPTZ) were purchased from Fluka (Germany). Folin-Ciocalteu reagent, ethanol, sodium

carbonate, di-sodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were all obtained from Merck (Germany). Ferrous chloride was obtained from QREC (Malaysia).

Sample Collection and Extraction

Total 10 commercially Larb seasoning were purchased from the local market in the north of Thailand. Ten gram of each sample was extracted with 200 ml of 95 % ethanol at 50 °C in a shaking water bath for 8 hours. The supernatants were stored in capped bottles and stored at -20 °C until use to determine total phenolic content, total flavonoid content and antioxidant activity.

Determination of Total Phenolic Content (TPC)

Total phenolic content of the extracts was determined by the Folin-Ciocalteau method with a minor modification from Singleton and Rossi [10]. Briefly, 20 µl of the extract, dissolved in ethanol was mixed with 100 µl of Folin-Ciocalteu's solution, then 80 µl of 7.5 % (w/v) sodium carbonate solution was added. The reaction mixture was incubated at room temperature in dark for 90 minutes and the absorbance was measured at 765 nm. Gallic acid was used as a standard and the total phenolic content was expressed as mg of gallic acid equivalents per gram dry weight of sample (mg GAE/100 g DW).

Determination of Total Flavonoids Content (TFC)

Total flavonoids content was determined according to the method of Mariniva *et al.* [2]. The extract 100 µl was mixed well with 400 µl of deionized water and 30 µl of 3 % sodium nitrite and stood for 5 minutes. Then, 30 µl aluminum chloride solution was added into the mixture, incubated for 6 minutes. After that, sodium hydroxide solution 200 µl was added and adjusted the volume with deionized water. The mixture was measured the absorbance at 510 mm. Catechin was used as a standard and the total flavonoids content was expressed as mg of catechin equivalents per gram dry weight of sample (mg CAE /100 g DW).

Determination of Antioxidant Activity

DPPH radical scavenging assay

The free radical scavenging activity was determined DPPH assay according to the method of Duan *et al.* [11]. The extract of 20 µl was mixed with 200 µl of 0.3 mM DPPH in methanol and then incubated at 37 °C for 30 min in the dark. Immediately, the absorbance was measured at 515 nm. Absorbance decreases were calculated as DPPH values by comparing with standard curves created by ascorbic acid, and the results were reported as mg ascorbic acid equivalent per gram dry weight of sample (mg ascorbic acid equivalent/100 g DW).

ABTS Radical Cation Decolorization Assay

ABTS radical scavenging activity was determined according to the method of Re *et al.* [12] with some modifications. The radical $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS and 2.45 mM potassium persulfate buffer (1:1) and kept for 16 h in dark at room temperature. After 16 hours, ethanol was used to adjust the absorbance of the radical $ABTS^{\bullet+}$ to 0.70 ± 0.05 at 734 nm. 1.9 ml of the radical $ABTS^{\bullet+}$ was added to 50 µl of sample at different concentrations. The mixture was stored at room temperature for 6 min, and then the absorbance was measured against the blank at 734 nm. The calibration curve was constructed using different concentration of ascorbic acid. The results were expressed in mg ascorbic acid equivalent/100 g DW.

Ferric Reducing Antioxidant Power Assay

The total antioxidant potential of a sample was determined using the ferric reducing ability of FRAP assay by Benzie and Strain [13] as a measurement of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which had increaseed the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (190 µl) and sample solutions (10 µl) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 15 min. Standard curve was prepared using different concentrations of $FeSO_4 \cdot 7H_2O$. All solutions were used on the day of preparation. The results were expressed in µmol Fe^{2+}/g DW. The standard curve of DPPH and ABTS (mg standard equivalent per 100 gram of dry weight sample) was constructed by the concentration of standard and percentage inhibition.

$$\% \text{ inhibiton} = \frac{(A._{\text{control}} 1\text{-}A._{\text{sample}})}{A._{\text{control}}} \times 100$$

Statistical Analysis

Data were presented as mean value ± standard deviation (three replicate experiments). Analysis of variance and significant differences among means were determined by one-way ANOVA using SPSS (version 17, SPSS Inc., Chicago, USA). Student's t-test and value of $p < 0.05$ were considered to be significant.

Results and Discussion

Total Phenolic and Flavonoid Content in the Larb Seasoning

Larb seasoning or Nam Prilk Larb usually composes of many culinary herbs and spices, which are interested for their content of phytochemicals important substance because of health-related benefits [14]. The antioxidant property in many culinary herbs and spices is related to the presence of phenolic compounds. The phenolic and flavonoids have been proven to increase the shelf life of foods as well as slowing the lipid, protein and enzymatic oxidation. Moreover, phenolic and flavonoid compounds reduce the rancidity development, which could prevent off-flavor of foods [2].

Total phenolic content (TPC) is an important parameter of total antioxidant capacity (TAC), and widely used for evaluation of herbs, spices, fruits, cereals and natural sources extract [3]. The Folin–Ciocalteu assay is based on the reduction of the Folin–Ciocalteu reagent by phenolic compounds under alkaline condition, the principle of assay is the reduction of the Folin-Ciocalteu reagent in the presence of phosphomolybdic/phosphotungstic acid complexes that are reduced to yield a blue-coloured chromophore with maximum absorption at 765 nm [3-4]. The commonly used reference standard is gallic acid [5]. Due to the diversity and complexity of natural mixtures of phenolic compounds in plant extracts, it is rather difficult to characterize every phenolic compound, thus the Folin-Ciocalteu assay, it is a simple and rapid method, widely used to determined the total concentration of phenolic compounds [6-7].

The total phenolic content (TPC) was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/ 100 g DW. Among all samples extracts investigated, total phenolic content ranged from 104.94 to 324.11 mg GAE/ 100 g DW (Table 1). The highest total phenolic content is the Nam Prilk Larb (No.10) which was collected from Chiang Mai area, because the main ingredient or seasoning components is it long peper, coriander seed and cardamom, nutmeg of but the lowest sample (No. 2) was collected from Lampang area. The total flavonoid content (TFC)

was determined in comparison with standard catechin and the results expressed in terms of mg CAE/ 100 g DW. The total flavonoid content of crude Nam Prilk Larb extracts were in the range of 35.01-253.31 mg CAE/ 100 g DW, respectively (Table 1). The highest and lowest total flavonoid content of Nam Prilk Larb was similar to the total phenolic content (No. 10 and 2, respectively).The total phenolic content was different because of the difference types of plant, geographic locations, environment, season, part used, processing and storage and plant extract contained lower total flavonoid content than total phenolic content, due to the presence of non-flavonoid phenolic substances in herbs and spices [7-15].

The phenolic compounds have been widely investigated to be responsible for the antioxidant activity of plant materials, also the potential models for developing the new primary antioxidants, which can prevent or delay *in vitro* and/or *in vivo* oxidation processes [16-18]. Flavonoids are the largest class of phenolic compounds, which are plentiful in the plants, and essential for human health because of their high pharmacological activities as radical scavengers [19-20]. Flavonoids are the one interested substances because of the health benefits, which it could arise the antioxidant activity, free radical scavenging capacity, coronary heart disease prevention and anticancer activity, while some flavonoids show the potential for anti-human immunodeficiency virus functions [21]. These results indicated that the phenolic and flavonoid compounds primarily contributed to the antioxidant capacity of Nam Prilk Larb. Moreover, it is apparent that Larb seasoning in Thai northern foods supplies antioxidant compounds.

Antioxidant Activity of the Extracts from Larb Seasoning

The determination of antioxidant capacity of natural materials *in vitro* as several analytical methods. The antioxidant activity of plant extracts or natural product extracts cannot be evaluated using only one method, because of the complex composition of phytochemical and oxidative processes [22]. Thus, the antioxidant activities of Nam Prilk Larb extracts were focused on three common assays, which are DPPH, ABTS and FRAP, were based on different radicals and mechanism of reactions.

Table 1 Total Phenolic and Flavonoid Content of Each Larb Seasoning Extracts.

Sample	Total phenolic content (mg GAE/100 g DW)	Total flavonoid content (mg CAE /100 g DW)
1	106.12 ± 3.08^{a}	50.34 ± 7.69^{b}
2	104.94 ± 4.69^{a}	35.01 ± 5.46^{a}
3	145.90 ± 3.35^{b}	66.93 ± 1.27^{c}
4	185.22 ± 5.46^{d}	123.94 ± 2.86^{e}
5	160.99 ± 3.90^{c}	64.33 ± 2.73^{c}
6	257.90 ± 1.89^{f}	113.46 ± 0.39^{d}
7	223.82 ± 5.23^{e}	111.23 ± 2.30^{d}
8	262.46 ± 6.69^{f}	165.12 ± 3.29^{g}
9	320.93 ± 5.38^{g}	134.37 ± 2.99^{f}
10	324.11 ± 2.89^{g}	253.31 ± 1.14^{h}

GAE: gallic acid equivalent; CAE: catechin equivalent

Mean (± S.D.) with different superscripts in each column are significantly different ($p < 0.05$).

a, b, c, d, e, f, g, h values in a column followed by different superscripts letters are significantly different ($p < 0.05$).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a single electron transfer (ET) reaction-based method with hydrogen atom transfer (HAT) reaction mechanism that has been widely used to determine the free radical scavenging ability of various sample [23-24]. The reaction is accompanied with color change of the DPPH from purple to yellow by the formation of DPPH upon absorption of hydrogen from an antioxidant, and the discolouration acts as an indicator of the antioxidant efficacy [20-21]. The DPPH activity of ten Nam Prilk Larb extracts are presented in Table 2. It was ranged from 142.44 to 745.59 mg ascrorbic acid/100 g DW ($p < 0.05$). Significant differences of DPPH activity among ten Nam Prilk Larb samples extracts were found ($p < 0.05$). The No.10 sample extract showed the strongest inhibition activity with 745.59 mg ascrorbic acid/100 g DW, followed by No.9 (549.11 mg ascrorbic acid/100 g DW) and No. 8 (505.85 mg ascrorbic acid/100 g DW), respectively.

The ABTS assay measures the ability of antioxidants to scavenge the stable radical cation $ABTS^{\bullet+}$(2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)), a blue-green chromophore with maximum absorption at 734 nm which decreases in its intensity in the presence of antioxidants [25]. The use of ABTS radicals for measuring radical quenching, redirecting antioxidants instead to distinguishing electron transfer reaction mechanism [3]. The ATBS activity of each Nam Prink Larb extract is shown in Table 2. The results of ABTS activity are not significantly different in the extract of 6^{th} – 10^{th} ($p > 0.05$). It was ranged from 460.06-465.04 mg ascorbic acid/100 g DW. The lowest ABTS activity (287.11 mg ascorbic acid/100g DW) is obtained from the extract of 1^{st} Nam Prilk Larb.

The FRAP assay determines the ability of the extracts to reduce ferric ions, which is the reducing potential of antioxidant reacting with ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex to the intensely blue colored ferrous tripyridyltriazine (Fe^{2+}– TPTZ) complex in acidic media [13, 25]. The ferric reducing ability powers of the extracts from Nam Prilk Larb sample expressed as FRAP values (μmol Fe^{2+}/g DW) are shown in Table 2. The No.6 sample extract had the highest FRAP value of 29.51μmol Fe^{2+}/g DW, followed by the 4^{th}, 1^{th} and 9^{th} (19.63, 17.22 and 17.19 μmol Fe^{2+}/g DW), respectively. Thus, the 6^{th} Nam Prilk Larb stands out as having significantly strongest FRAP activity ($p < 0.05$).

The antioxidant activities of Nam Prilk Larb are shown in Table 2. The phenolic compounds and flavonoids are mainly contributed in herbs and spices, which are consisted in Nam Prilk Larb. Generally, Thai culinary herbs, spices or vegetables have been recognized to contain excellent source of antioxidant compounds, which contain high phenolic compounds content, e.g., gallic acid, caffeic acid, catechins, anthocyanin, quercitin and rutin [6]. They could inhibit oxidative stress by antioxidant mechanism which are known to be a powerful chain breaking antioxidant, radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers, is acting as primary antioxidants or free radical terminators, so it was widely to evaluate their content in plant extracts [6, 26].

Table 2 Antioxidant Activities of Each Larb Seasoning Extracts.

Sample	DPPH (mg ascorbic acid/ 100g DW)	ABTS (mg ascorbic acid/ 100g DW)	FRAP (μmol Fe^{2+}/g DW)
1	205.43 ± 1.20^{b}	287.11 ± 4.40^{a}	17.22 ± 0.34^{c}
2	142.44 ± 5.99^{a}	290.54 ± 5.24^{a}	13.06 ± 0.76^{a}
3	279.10 ± 9.24^{c}	420.27 ± 5.00^{b}	16.67 ± 0.86^{d}
4	384.55 ± 3.34^{e}	449.44 ± 3.19^{c}	19.63 ± 0.68^{c}
5	298.59 ± 2.47^{d}	460.06 ± 3.96^{d}	17.14 ± 0.96^{d}
6	492.67 ± 7.89^{f}	465.04 ± 1.29^{d}	29.51 ± 1.05^{e}
7	485.22 ± 2.53^{f}	463.08 ± 1.04^{d}	14.33 ± 0.61^{b}
8	505.85 ± 1.86^{g}	462.14 ± 0.68^{d}	14.89 ± 0.69^{b}
9	549.11 ± 6.59^{h}	462.69 ± 1.07^{d}	17.19 ± 0.19^{c}
10	745.59 ± 4.00^{i}	465.01 ± 4.01^{d}	14.36 ± 0.09^{b}

Mean (± S.D.) with different superscripts in each column are significantly different ($p < 0.05$).

a, b, c, d, e, f, g, h, i values in a column followed by different superscripts letters are significantly different ($p < 0.05$).

Conclusion

Nam Prilk Larb is consists of various herbs and spices. The antioxidant activity by three different methods DPPH radical scavenging assay, ABTS radical cation decolorization assay and Ferric reducing antioxidant power assay. The result showed that 10 th Larb seasoning had total phenolic and total flavonoid contents higher 324.11 mg GAE/100 g DW and 253.31 mg CAE/100 g DW where as antioxidant capacity was 745.59 mg ascorbic acid/100 g DW for DPPH. The highest ABTS and FRAP activity was obtained from the crude extract of 6 th Larb seasoning, which was 465.04 mg ascorbic acid/100 g DW and 29.51 μmol $Fe2^{+}$/g DW, respectively. Moreover, the total phenolic and total flavonoid contents of each sample were also determined and related to their antioxidant activity.

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