Screening of Antioxidant Activity, Total Phenolics, and Flavonoid on Selected Northern Thailand Medicinal Rhizomes

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Abstract. The study aimed to determine the freeze-dried chemical and physical properties of northern Thailand medicinal rhizomes for potential use in food and pharmaceutical products. Those are *Kaempferia parviflora, Alpinia galanga* and *Boesenbergia rotunda*. Freeze-drying was chosen as an efficient method to preserve the high-antioxidant materials. Several analyses were conducted to determine the potential of these rhizomes. Determining antioxidant activity using two methods from the DPPH and ABTS radical scavenging activity, Total Phenolic was using Folin Ciocataleu and Total Flavonoid assay. The analysis results showed that the highest antioxidant activity scavenges activity was *Boesenbergia rotunda* in the DPPH scavenging activity. At the same time, the results of ABTS scavenging activity also found similar results: *Boesenbergia rotunda* showed the highest inhibition compared to other rhizomes. Meanwhile, total phenolic and flavonoid remained the same, with the highest being *Boesenbergia rotunda*. In conclusion, among the selected northern Thailand rhizomes, *Boesenbergia rotunda* becomes the potential rhizome to be conducted to optimise extraction for a further process and apply both food and pharmaceutical products.

Keywords: *Boesenbergia rotunda,* Bioactive compounds, Antioxidant, Phenolic, Flavonoid.

1 Introduction

Historically, medicinal plants are an essential source of bioactive substances. In rural places like northern Thailand, various widely accessible and less expensive plants are utilised as traditional medicines. Plants generally produce many secondary metabolites (compounds related to groups like phenol, alkaloids, terpenoids, and glycosides), constituting an essential source of microbicides, pesticides, fungicides, and many pharmaceutical products [1]. Moreover, those bioactive compounds can also be applied in developing food products and as functional food [2]. Members of the Zingiberaceae family are found to be a rich source of the substance of phytochemical interest. They are rich in curcuminoids and recognised for their broad spectrum of biological activities

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spectrum of biological activities and bioactive compounds, such as phenolic compounds, which vary in chemical structures, physicochemical characteristics, and functional properties [3]. The number of plants from this family is used in traditional medicine because of its broad spectrum of pharmacological activities [4].

Polyphenols, or phenolic compounds, are among plants' most common and widely distributed families of secondary metabolites, with approximately 8000 phenolic compounds now recognised [5]. The complexity of natural polyphenols presents a range, covering simple molecules such as phenolic acids, phenylpropanoids, and flavonoids, as well as highly polymerised compounds like lignins, melanins, and tannins. Among these, flavonoids are the most abundant and extensively distributed subclass [6]. Polyphenolics exhibit a variety of biological activities, including anti-inflammatory, antibacterial, anticarcinogenic, and anti-allergic, antiviral [7].

Some rhizomes are traditionally used as medicine and food additives. One of them is *Kaempferia parviflora*, also known as black ginger, which is popular and traditionally used as an alternative medicine to counter a variety of diseases, including allergic, inflammation, ulcers, gout, colic disorders, abscesses, allergies, and osteoarthritis [8]. In addition, *Alpinia galanga* is also commonly used in Thai food. This rhizome contains volatile essential oils and other chemicals with tremendous medicinal value and is in high demand among traditional healthcare practitioners [9]. Simultaneously, *Boesenbergia rotunda* was frequently used as a culinary component and traditional treatment for various ailments, including aphthous ulcers, xerostomia, gastric distress, leucorrhoea, and dysentery [10]. To get the optimum conditions from the rhizomes, pre-treatment such as drying to maintain the bioactive compounds inside. As mentioned in the previous study, freeze-drying was chosen in this research, which is more efficient than another drying method. Freeze drying has been proven to preserve the total phenolic content and carotenoids, which also had higher antioxidant activity in ginger [11]. Besides that, it is to maintain the same conditions for all the rhizomes and extend the shelf-life. This study aimed to determine and evaluate the bioactive compounds contained in the three medicinal plants commonly used in the northern part of Thailand using freeze-drying as a pre-treatment.

2 Methodology

2.1 Preparation of Medicinal Rhizomes Extracts

The three rhizomes (*Kaempferia parviflora, Alpinia galanga*, and *Boesenbergia rotunda*) were bought at the CMU Agri Shop in Chiang Mai University. They were kept in the Department of Food Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand. Those rhizomes were cleaned, sliced for 2mm thickness, and stored in the freezer before drying. The drying method used freeze drying with conditions ranging from -40℃ to 40℃ with 20Pa for 24 hours. The dried rhizome was then ground into small particles and sieved using a steel mesh sieve of 0.3mm. Ten grams of each powdered rhizome was extracted using the maceration method, which soaked for 72 hours using ethanol (EtOH) 95%. The extract was subsequently filtered using cheesecloth and Whatman filter paper No. 1, and then the residue was centrifuged at 5000 rpm for ten minutes to get another extract. The

extracts were evaporated using a Rotary evaporator to remove the solvent in the extracts. All the extract was placed at -4℃ until further analysis.

2.2 Physicochemical Analysis

Moisture and ash content analyses were performed to assess the physical properties of powdered rhizomes. These parameters were determined following AOAC methods 927.05 and 930.30 [12].

2.3 Powder Properties

The method was followed from [13] with a few modifications to the sample size. Ten grams of powder were meticulously measured and placed into a 100-millilitre graduated cylinder. Determining bulk density involved utilizing the measured volume obtained from the cylinder and the mass-to-volume ratio. The method to calculate the tapped density of powders, the cylinder underwent 120 taps, and the associated sample volume was recorded. Powders' flowability and cohesiveness were tested using the Carr index (CI) and Hausner ratio (HR).

Table 1. Flowability specifications for Carr Index and Hausner Ratio.

Flowability	Carr Index (CI) %	Hausner Ratio
Excellent	0-10	1.00-1.11
Good	11-15	1.12-1.18
Fair	16-20	1.19-1.25
Passable	21-25	1.26-1.34
Poor	26-31	1.35-1.45
Very Poor	32-37	1.46-1.59
Very, Very Poor	>38	>1.60

Determining the coefficients of interparticle contact (CI) and Hausner ratio (HR) required estimating these values using the measurements obtained from the bulk and tapped densities of the powder. This calculation process is outlined in Equations (1) and (2).

$$CI = \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} \times 100 \quad (1)$$

$$HR = \frac{\rho_{tapped}}{\rho_{bulk}} \quad (2)$$

2.4 Colour Analysis

The portable colorimeter (Konica Minolta Chroma Meter CR-400 Japan) was performed on the sample's surface, employing the Cie Lab color system (L* a* b*).

The instrument was calibrated using the white-colored disc (L* = 98.82, a* = -0.07, and b* = -0.45) supplied with the instrument before analysis. The readings were taken in triplicates. The L* (brightness or whiteness), a* (redness and greenness) and b* (yellowness and blueness). The calibration of the colorimeter was performed by utilizing a standard white plate. The CIE L*, a*, and b* values were acquired and afterward used to construct the whiteness index (WI) according to the methodology described by [14], utilizing equation (3).

$$WI = 100 - \sqrt{(100 - L^{*})^{2} + (a^{*^{2}} + b^{*^{2}})} \quad (3)$$

2.5 Antioxidant Analysis

Determination 1,1-diphenyl-2-picryhydrazyl (DPPH) Radical-Scavenging Assay. The DPPH scavenging activity was determined as described by [15] with some modification. A 0.1 mM solution of ethanol DPPH will be prepared. 1 ml of 0.1 mM DPPH in ethanol will be added to 1 mL of each sample with different concentrations of 3 ml of ethanol and incubated at ambient temperature for 30 minutes in the dark. The experiment will be repeated three times. The DPPH for free radical scavenging ability will be given as the Trolox equivalent. The DPPH radical scavenging activity was calculated by the following equation (4).

$$= 100 - [(ABS_{sample} - ABS_{blank}) \times 100]/(ABS_{control}) \quad (4)$$

Where ABS_{sample} is the absorbance of the presence of a sample extract, ABS_{blank} is the absorbance without sample only ethanol, and $ABS_{control}$ is the absorbance of DPPH without any sample extracts.

Determination of Radical Scavenging Activity ABTS. The ABTS scavenging assay was performed as mentioned by [16] with minor modifications. Briefly, a mixture of 5 ml of 7.4mM 2,2'-asino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and 5 ml of 2.6 mM potassium persulphate was kept in the darkroom at room temperature for 12-18 hours to generate the ABTS+ reaction solution. The ABTS+ solution was added (2850µL) to 150 µL of the diluted extracts and mixed well. Trolox was prepared in 95% ethanol for standard use and analysed in equal conditions. The findings were quantified using the Trolox equivalent antioxidant capacity (TEAC), measured in mM Trolox equivalents

2.6 Phenolic Content Assay

Total phenolics will be determined using Folin-Ciocalteu's reagent, adapted from [17]. A volume of 10 µL of the sample solution was combined with 100 µL of commercially available Folin-Ciocalteu's reagent and 1580 µL of water. Following a short period of incubation at ambient temperature lasting 5 minutes, a volume of 300

µL of sodium carbonate in a saturated phase will be given. The blue color was generated and read at room temperature at 760 nm after two hours using a UV-Vis spectrophotometer. The calibration curve used gallic acid solution with different concentrations. Total phenolic content was expressed as Gallic Acid equivalent (GAE).

2.7 Flavonoid Content Assay

Total flavonoid content was quantified using a well-established approach from previous studies [18] with minor modifications. An aliquot of 0.1 ml of extracts or standard (0.1, 0.5, 1.0, 2.5, and 5mg/ml) was added into the conical tubes with an additional 500 µl of distilled water mixed with 100 µl of 5% Sodium Nitrate ($NaNO_3$). Following a time interval of 6 minutes, a volume of 150 µl of a solution containing 10% Aluminium Chloride ($AlCl_3$ $6H_2O$) was introduced and left unchanged for 5 minutes. Subsequently, 200 µl of a solution containing 1M Sodium Hydroxide (NaOH) was added. The solution was well mixed and stayed for 15 minutes. The absorbance of the combination was measured at a wavelength of 510 nm using a UV spectrophotometer. The total flavonoid content was estimated as Quercetin equivalent (QE).

2.8 Statistical Analysis

The statistical studies were performed using the Minitab 21.3 statistics program. The results obtained were analyzed using analysis of variance (ANOVA), and any variations between the means were determined using Tukey's multiple comparison test. The statistical significance level was set at $p<0.05$. The results are reported as the Mean ± the standard deviation based on three replications.

3 Results and Discussions

3.1 Powder Characteristics

The initial moisture content of *Boesenbergia rotunda*, *Alpinia galanga*, and *Kaempferia parviflora* was 91%, 87.6%, and 63%, respectively. To ensure quality preservation and prevent sample deterioration, freeze-drying was chosen as the preferred method to reduce moisture content in the sample. The data shows a significant reduction of moisture in each sample. In *Boesenbergia rotunda,* the decreasing value was 82.02%, followed by *Alpinia galanga at* 77.6% and 58.87% at *Kaempferia parviflora.*

Table 2. Moisture and Ash content of powdered selected rhizomes.

Sample	Moisture Content	Ash Content
Boesenbergia rotunda	8.98 ± 0.09^{b}	88.77 ± 0.15^{c}
Alpinia galanga	10.00 ± 0.6^{a}	93.28 ± 0.08^{b}

Kaempferia parviflora	4.13+016[c]	94.88±0.04[a]

Table 3. Flowability powder of rhizomes.

Sample	Bulk density	Tapped density	Carr Index	Hausner ratio	Flowability
Boesenbergia rotunda	0.18±0.01[b]	0.30±0.03[b]	42.13±2.14[a]	1.73±0.03[a]	Very, very poor
Alpinia galanga	0.18±0.01[b]	0.27±0.01[c]	33.82±2.48[b]	1.51±0.06[b]	Very poor
Kaempferia parviflora	0.43±0.03[a]	0.62±0.06[a]	30.08±1.93[c]	1.43±0.04[c]	Poor

The flowability of three selected rhizome freeze-dried was measured based on the Carr index and Hausner ratio. The Carr index was 30.0 to 42.1, with *Boesenbergia rotunda* having the highest value, followed by *Alpinia galanga* and *Kaempferia parviflora*. At the exact moment, the Hausner ratio had similar results as the Carr index, in the range of 1.43 to 1.73, with the same sequence for the highest and lowest values. Based on the flowability specification of the Carr index and Hausner ratio, the flowability results showed that the freeze-dried selected rhizome had flowability characteristics from poor to very, very poor. The drying method employed had a pronounced impact on the flow properties of the powders. Specifically, freeze-dried powders demonstrated notably low actual and bulk densities, indicative of their lightweight nature. Despite this, they exhibited high levels of porosity and solubility, suggesting their potential for rapid dissolution and absorption. Furthermore, the colour assay results revealed distinct colour profiles for each rhizome. Notably, *Boesenbergia rotunda* exhibited the lightest colour compared to the other rhizomes, hinting at variations in the concentration and composition of bioactive compounds among them. This underscores the importance of considering colour characteristics as indicators of the presence and abundance of bioactive compounds within the rhizomes.

Table 4. Colour assay of powdered selected rhizomes.

Sample	L*	a*	b*
Boesenbergia rotunda	68.62 ±0.35[b]	3.33±0.10[c]	18.76±0.08[a]
Alpinia galanga	75.12±0.19[a]	4.27±0.11[b]	16.67± 0.06[b]
Kaempferia parviflora	54.53±0.39[c]	6.71±0.06[a]	-0,83±0.17[c]

3.2 Antioxidant Scavenging Activity

The assessment of antioxidant activity yielded notable results, ranging from 0.61 to 52.50 mg/ml for DPPH radical scavenging activity and from 0.97 to 6.44 mg/ml for ABTS scavenging activity, as depicted in Table 5. *Boesenbergia rotunda* exhibited the lowest scavenging activity in both assays, with *Kaempferia parviflora* following suit. Conversely, Alpinia galanga demonstrated the highest scavenging activity among all samples. These findings underscore the variability in antioxidant potency across the tested rhizomes, with Alpinia galanga emerging as the most effective scavenger of free radicals compared to *Boesenbergia rotunda* and *Kaempferia parviflora*.

Table 5. Antioxidant scavenging activity of rhizomes.

Sample	DPPH Scavenging activity (mmol/g) TEAC	ABTS Scavenging activity (mmol/g) TEAC
Boesenbergia rotunda	0.55±1.25[c]	6.44±0.06[c]
Alpinia galanga	0.35±1.36[b]	1.92±0.47[b]
Kaempferia parviflora	0.07±0.22[a]	6.44±0.06[a]

The analysis of the results revealed the presence of potent antioxidant compounds in all three rhizomes, capable of effectively scavenging free radical ions. The disparity between the DPPH and ABTS assays lied in their colour reactions, with DPPH producing a purple colour and ABTS yielding a blue-green hue. Furthermore, the ABTS assay offered the advantage of distinguishing between hydrophilic and hydrophobic antioxidant capacities, as it remained unaffected by ionic strength variations. Additionally, these findings were correlated with the results of phenolic and flavonoid content assays, providing insights into the levels of bioactive compounds present in the three rhizomes.

3.3 Phenolic and Flavonoid content.

Phenolic acids represent a class of organic compounds characterized by a benzene ring, carboxyl group, and hydroxyl group. The antioxidant effectiveness of phenolic acids depends on the number of hydroxyl groups present in their molecular structure and their steric properties. The position and type of substitution on the aromatic ring influence the antioxidative properties of these compounds. According to the phenolic content analysis from selected rhizomes (Table 6), *Boesenbergia rotunda* exhibits the highest phenolic content, which correlates with its antioxidant scavenging activity. Following *Boesenbergia rotunda*, *Alpinia galanga* demonstrates a phenolic content of 225.49 mg GAE/g, while *Kaempferia parviflora* exhibits the lowest phenolic content among the tested rhizomes, with 174.40 mg GAE/g.

Table 6. Total phenolic and flavonoid content of rhizomes.

Sample	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Boesenbergia rotunda	314.03±16.0[a]	119.32±4.89[a]
Alpinia galanga	225.49±4.16[b]	49.89±0.53[b]
Kaempferia parviflora	174.40±2.69[c]	20.48±1.67[c]

A Flavonoid assay was conducted to observe the flavonoid content in the selected rhizomes. The result showed a significant amount of flavonoid of *Boesenbergia rotunda*, which had 119.32 mg QE/g, followed by *Alpinia galanga* and *Kaempferia parviflora,* 49.89 and 20.48 QE/g. The amount of flavonoid in the result was in line with the other analysis: antioxidant assay and total phenolic content.

3.4 Various of Time Extraction

Table 7. TPC and TFC of *Boesenbergia rotunda* with various time extraction

Sample	Time	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Boesenbergia rotunda	40	276.16±7.36	46,47±2.98
	60	307.20±37.62	20.64±0.79
	80	299.01±37.04	23.66±0.31

The results obtained from the previous table reveal that *Boesenbergia rotunda* exhibits the highest levels of bioactive compounds, including phenolic and flavonoid content. To further optimize extraction conditions, the length of extraction time was varied. The analysis revealed that the highest total phenolic content was achieved after 60 minutes of maceration, with a value of 307.20±37.62 expressed in gallic acid equivalent. However, no significant difference in total phenolic content was observed across different extraction time variations. In contrast, the highest flavonoid content was obtained after 40 minutes of maceration, with a value of 46.47±2.98 expressed in quercetin equivalent.

4 Conclusions

The study indicates variations in the antioxidant compound contents among commonly consumed rhizomes in Thailand. Certain plants emerge as promising natural antioxidant sources due to their extracts exhibiting high antioxidant activity. *Boesenbergia rotunda* demonstrates the highest activity, followed by *Alpinia galanga* and *Kaempferia parviflora*, respectively. Analysis reveals a correlation between the phenolic and flavonoid contents of the tested plants and their antioxidant potency. These findings suggest that the antioxidant activities of these plants may be attributed to their chemical constituents. Further research is warranted to explore the potential of these rhizomes, including optimization of extraction methods, application of extracts, and investigation of the compounds within the extracts, particularly in *Boesenbergia rotunda*.

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