



## Study of active compounds in Zingiberaceae and Niacin content in Broken Rice for herbal soap formulation

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### Abstract

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This research studied the chemical composition of three species from the Zingiberaceae family (*Curcuma longa* L., *Curcuma aromatica* Salisb. and *Zingiber montanum* (J.Koenig) Link ex A.Dietr.) and analyzed the niacin content in extracts from four cultivars of broken rice, namely Daeng Mueang Loei, Siw Kliang Mueang Loei, Black Sticky Rice and Sticky Rice RD6, for use as active ingredients in the development of herbal soap formulations, as an option to add value to the rice farming community of Ban Nam Yen, Dan Sai District, Loei Province. The study of active compounds Zingiberaceae using HS-GCMS revealed that *C. longa* L. and *Z. montanum* (J.Koenig) Link ex A.Dietr.) contained Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- (*cis*-sabinene) as the highest component, while *C. aromatica* Salisb. had (R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene (*beta*-Curcumene) as the predominant compound. Quantitative analysis of curcumin by UV-Visible spectrophotometry showed that *C. longa* had the highest curcumin content at  $2.92 \pm 0.13$  g 100g<sup>-1</sup>. For the analysis of niacin content in broken rice by HPLC, Daeng Mueang Loei was found to have the highest content at  $5.69 \pm 0.19$  mg kg<sup>-1</sup>, whereas niacin was not detected in Black Sticky Rice. Method validation confirmed reliability for the quantitative analysis ( $R^2 \geq 0.9950$ , %RSD  $\leq 11\%$ , 80-110% recovery). Formula 1 demonstrated superior physical and chemical properties in the base formula, identifying it as suitable for herbal soap development. These results establish foundational data for developing products incorporating rice and Zingiberaceae extracts.

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### 1. Introduction

The use of herbs in cosmetic products has continuously gained popularity, especially plants from the Zingiberaceae family which are rich in active compounds with anti-inflammatory and antioxidant properties. These compounds enhance skin nourishment and smoothness while promoting radiance. Consequently, skincare formulations incorporating bioactive ingredients, particularly natural antioxidants that restore skin health, are increasingly sought after in cosmetic science [1]. The shift in consumer behavior towards products that align with sustainability, health-consciousness, and environmental friendliness has led to continuous growth of the natural cosmetics and skincare market. Market reports show that

consumers worldwide are increasingly seeking safe and effective natural alternatives. This growing demand has stimulated research and the development of products incorporating bioactive compounds from local plants. Soap is an essential cosmetic product used daily for basic skin cleansing, making it an important channel for delivering beneficial active compounds and ingredients to the skin. The development of soap formulations with properties beyond cleansing, such as enhancing effectiveness by adding substances that help nourish the skin, is one approach to product development that responds to consumer demands. Rice bran, which is a by-product from the rice milling process that is often overlooked and has low commercial value, has been found to contain interesting levels of niacin

(Vitamin B3). Niacin is a water-soluble vitamin that is essential for the function of many enzymes in the body. When used as an ingredient in cosmetic products, it has properties that help to even skin tone, reduce dullness, diminish fine lines, help brighten the skin and to stimulate the skin to produce collagen and ceramides. Previous studies have found that using products with niacin at a concentration of 5% can effectively reduce wrinkles and dark spots after continuous use for 12 weeks [2]. Meanwhile, curcumin is a principal compound present in extracts of Zingiberaceae plants. It is responsible for their characteristic yellow-orange coloration. This compound exhibits bioactive properties including skin anti-inflammation, UV damage repair and anti-acne bacterial inhibition. This dual functionality positions them as versatile cosmetic ingredients [3, 4]. Moreover, studies have reported that curcumin can stimulate collagen production in the skin, thereby improving its elasticity and strength. This contributes to a youthful appearance and reduces the appearance of wrinkles [5]. Curcumin also exhibits antibacterial properties, particularly against *Propionibacterium acnes* and *Staphylococcus epidermidis* which are major causes of acne. This gives it potential for development into products for acne-prone skin [6]. This study investigated the active compounds in the Zingiberaceae family and the niacin content in Loei broken rice for herbal soap formulation. The results of this study can serve as a guideline for developing soap formulations containing important natural ingredients that are safe for consumers, benefiting both the industry and the long-term health of users.

## 2. Materials and Methods

### Plant materials

Samples of three Zingiberaceae species, namely *Curcuma longa* L., *Curcuma aromatica* Salisb. and *Zingiber montanum* (J.Koenig) Link ex A.Dietr. were collected from Huaytad village, Na Dok Kham Sub-District, Na Duang District, Loei Province. Four cultivars of broken rice, specifically, Daeng Mueang Loei, Siw Kliang Mueang Loei, Black Sticky Rice and Sticky Rice RD6 were collected from Ban Nam Yen, Dan Sai District, Loei Province. Voucher specimens were deposited in the Biology Program, Department of Science, Faculty of Science and Technology, Loei Rajabhat University.

### Chemicals and reagents

Curcuminoids (mixed standard; purity  $\geq$  95%) were obtained from Sigma-Aldrich (USA). Nicotinic acid (vitamin B3; analytical standard grade; purity  $\geq$  99.5%) and analytical-grade solvents, including methanol and ethanol (AR grade), were purchased from Merck (Germany). Additional reagents included distilled water, phosphate buffer (20 mM, pH 3), glycerin (USP grade, 99.7%, food grade), and potassium N-cocoyl glycinate (cosmetic-grade amino surfactant).

### Instruments

Gas chromatography–mass spectrometry (GC-MS) was performed using a single quadrupole GC/MS system (Agilent Model 5977-2100 cV6, USA). High-performance liquid chromatography (HPLC) was carried out with a KNAUER AZURA DAD 6.1L system. UV-visible spectrophotometric analysis was conducted using a Lambda 25 UV-Vis spectrophotometer. A rotary vacuum evaporator (Model R-124) from Buchi Labortechnik AG (Switzerland) was used for sample concentration.

### Analysis of active compounds in the Zingiberaceae family using HS-GCMS

The analysis of active compounds was performed on samples of Zingiberaceae using GC/MSD in conjunction with MassHunter Data Analysis software. Sample preparation was automated by placing approximately 2.0 g of the sample into a headspace vial, followed by incubation at 80°C for 20 minutes. The injection needle temperature was set to 120°C, and the injection mode was split, depending on substance concentration. The gas chromatography process began with an oven temperature of 50°C, maintained for 2 minutes, and gradually increased by 5°C per minute up to 200°C, where it was maintained for 5 minutes. The injector temperature was set to 250°C with a split ratio of 10:1. The separation of compounds was achieved using a DB-5MS column or equivalent (30 m  $\times$  0.25 mm I.D.  $\times$  0.25  $\mu$ m film thickness) with helium as the carrier gas, and a flow rate of 1.0 mL min<sup>-1</sup>. In the mass spectrometry analysis, the ionization mode was Electron Ionization (EI) at 70 eV, the ion source temperature was set to 230°C and the quadrupole temperature was set to 150°C. Scanning was performed in the m/z range of 35-500. Chromatographic data was processed using mass spectrometry (MS) and peaks were identified and classified based on retention time with a library search comparison using NIST17.L Version 10.

### Quantitative analysis of curcumin in the Zingiberaceae family

The sample was dried in an oven at 55°C until a constant weight was reached, then ground to a fine powder and sieved through a 20-mesh sieve. 0.30 g of the prepared sample was weighed and 5 mL of 95% ethanol was added. The mixture was stored at room temperature for 24 hours and then filtered through no. 93 Whatman filter paper. The volume of the extract was adjusted to 10 mL. The curcumin content was analyzed using a UV-Visible Spectrophotometer at a wavelength of 427 nm and the curcumin concentration was calculated by comparison with a standard curcuminoids solution [7].

### Quantitative analysis of niacin (vitamin B3)

A total of 0.10 g of each broken rice variety was weighed. Methanol–water solution was added to bring the total volume to 10 mL. The mixture was thoroughly combined to obtain a homogeneous solution. The mixture was subjected to ultrasonic extraction for 10 minutes.

The extract was filtered through Whatman No. 93 filter paper, then filtered again using a 0.45  $\mu\text{m}$  filter. The final volume of the filtrate was adjusted to 10 mL. The niacin content of the prepared sample was analyzed using the conditions outlined in Table 1, and compared with a standard niacin solution.

**Table 1** Optimized chromatographic conditions for the proposed HPLC method.

Parameter	Chromatographic Conditions
Mobile phase	phosphate buffer 20 mM pH 3.0 : methanol (40:60)
Column	reversed-phase C18 column (250 mm $\times$ 4.6 mm I.D., 5 $\mu\text{m}$ particle size)
Flow rate	0.7 mL min $^{-1}$
Detector	UV Detector
Detection Wavelength	260 nm
Injection volume	20 $\mu\text{L}$

#### Analytical Method Validation

The results were validated by the analytical method according to the standards of the International Conference on Harmonization: ICH (2005) and AOAC International (2002).

#### Linearity and Calibration Curve

The linearity of the method was evaluated by constructing calibration curves at five concentration levels. Standard solutions of niacin and curcuminoid in the concentration range of 1–250  $\mu\text{g mL}^{-1}$  were prepared, with each concentration analyzed in five replicates ( $n=5$ ). HPLC determination was performed, and 20  $\mu\text{L}$  of the solution was injected under the operating chromatographic conditions described above. The calibration graph was constructed by plotting the peak area versus the concentration of curcuminoid and niacin. The correlation coefficient ( $R^2$ ) and regression equation were calculated.

#### Precision

The intra-day precision was determined by analyzing a standard solution containing 2 and 10  $\mu\text{g mL}^{-1}$  of curcuminoid and niacin seven times within one day, while the inter-day precision was examined over three consecutive days using the proposed method. The precision was expressed as the percent relative standard deviation (% RSD) as shown in equation (1);

$$\% \text{RSD} = \frac{SD \times 100}{\bar{x}} \quad (1)$$

Where SD means Standard Deviation  
 $\bar{x}$  means Theoretical value

#### Accuracy (%Recovery)

Recovery was used to evaluate the accuracy of the method. Standard addition was performed with the pre-analyzed sample solution, and 5 replicate analyses were conducted. The percent recovery was then calculated as shown in equation (2);

$$\% \text{Recovery} = \frac{a \times 100}{b} \quad (2)$$

Where  $a$  means Experimental value  
 $b$  means Theoretical value

#### Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ under the proposed chromatographic conditions were determined by diluting the working standard solution to the lowest concentration of analytes at 3:1 and 10:1, for LOD and LOQ respectively as shown in equations (3-4);

$$\text{Limit of Detection (LOD)} = 3 \times \text{SD} \quad (3)$$

$$\text{Limit of Quantification (LOQ)} = 10 \times \text{SD} \quad (4)$$

Where SD means Standard Deviation

#### Development of soap formulations from *C. longa* and Daeng Mueang Loei extracts

Three soap formulations were developed using extracts from *C. longa* and Daeng Mueang Loei extracts, as detailed in Table 2. A glycerin soap base was weighed into a 250 mL beaker and melted in a water bath at 90 °C, while being gently stirred in a consistent direction until fully melted. The temperature was then reduced to 60 °C. Afterwards, one gram of honey, *C. longa* and Daeng Mueang Loei extracts, and amino bubble were added. The mixture was then stirred gently in the same direction. Once 1 gram of fragrance had been incorporated, the mixture was poured into silicone molds and left to solidify. After the soap had hardened, it was removed from the molds, shrink-wrapped to prevent surface moisture formation and appropriately packaged.

**Table 2** Formulation of developed soap containing herbal extracts.

Ingredients (grams)	Formula		
	Control Formula	Formula 1	Formula 2
Soap Base	60	60	50
Water	10	-	-
Daeng Mueang Loei Extract	-	5	10
<i>C. longa</i> Extract	-	5	10
amino bubble	3	3	3
Honey	1	1	1
Fragrance	1	1	1

### Study of the physical and chemical stability of soap pH Testing

One gram of finely ground soap was weighed and dissolved in distilled water. The volume was adjusted to 50 mL. Next, the pH meter was calibrated with buffer solutions of pH 4.0, pH 7.0, and pH 10.0. After calibration, the pH of the soap solution was measured using the pH meter. The electrode was immersed into the solution, and the pH value was recorded once it stabilized.

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### Foam formation testing

One gram of soap sample was dissolved in 100 mL of distilled water. Foam formation was tested in tap water by pouring the soap solution into a beaker containing 100 mL of tap water. A stirring machine with a fixed speed was used for 1 minute. The amount of foam formed was recorded, and the stability of the foam was checked every 1 minute until the foam dematerialized. The experiment was repeated by replacing tap water with distilled water.

### Water solubility testing

The water solubility test was performed by cutting 5 grams of soap and placing it in 100 mL of distilled water at room temperature for 30 minutes. The amount of soap dissolved in water was determined by removing the soap, drying it thoroughly and reweighing it to assess the weight change after the test.

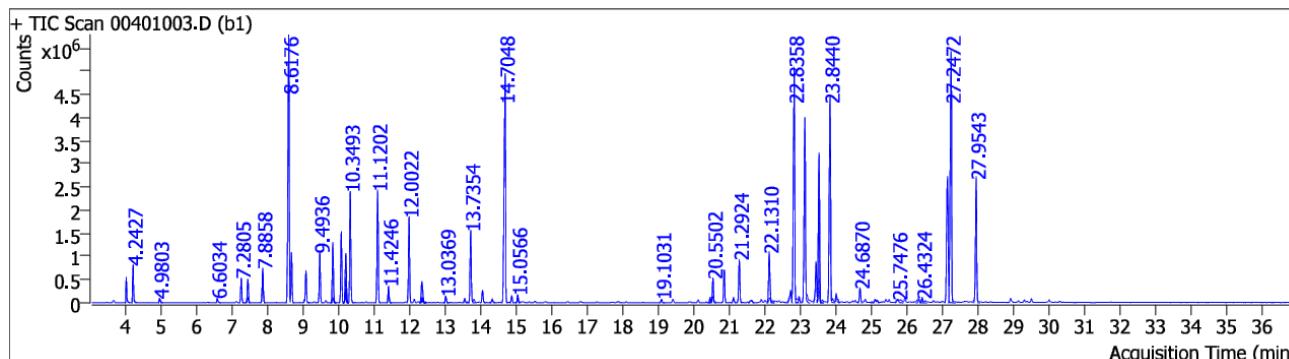
### Statistical analysis

Statistical tests used in this evaluation and study include accuracy, precision, linearity, range, limit of detection (LOD), limit of quantitation (LOQ) and related statistics for method validation, such as the mean ( $\bar{x}$ ) and standard deviation (SD), with five replicate determinations.

## 3. Results and Discussion

### Active compounds in Zingiberaceae family

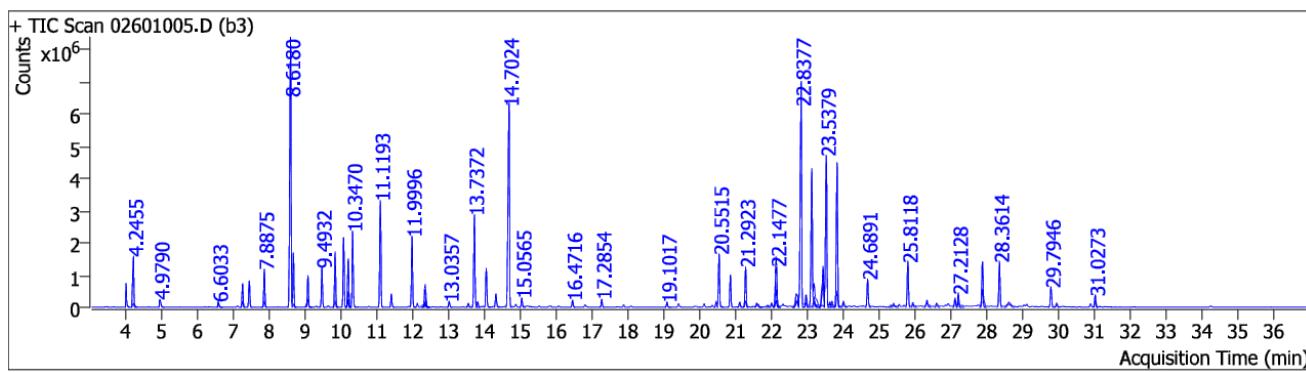
The chemical composition of *C. longa*, *Z. montanum* and *C. aromatic*a in the Zingiberaceae was determined using Headspace Gas Chromatography–Mass Spectrometry (HS-GC/MS) techniques. The results revealed several bioactive compounds (Figs.1-3). The chromatographic profiles were identified based on retention time and mass spectral database (NIST17 Library) with Match Factor values of 80% or higher, as shown in Figs. 1-3 and Table 3-5. In the *C. longa* sample, several major compounds were identified, for example Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl)-(cis-sabinene, RT= 8.6176 min, Area%-T = 9.33) and Turmerone (RT= 27.242 min, Area%-T = 9.95). This is consistent with previous studies indicating its pharmacological activity, particularly its anti-inflammatory and antioxidant properties [8, 9]. The study results of *Z. montanum* revealed major chemical components such as Bicyclo[3.1.0] hexane, 4-methylene-1-(1-methylethyl)-(cis-sabinene, RT= 8.6180 min, Area%-T = 12.63) and Terpinen-4-ol (RT= 14.7024 min, Area%-T = 10.66). In addition, the detection of *cis*-sabinene, epicurzerenone, and neocurdione at the highest levels is consistent with previous reports indicating anti-inflammatory and analgesic properties, supporting the medicinal value of *C. aromatic*a [10]. The analysis of *C. aromatic*a revealed major components, including (R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene (*beta*-Curcumene, T=23.6041 min, Area%-T = 28.42) and Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-(*alpha*-Curcumene, RT= 22.8748 min, Area%-T = 21.48) as well as other sesquiterpene and monoterpene compounds. Amongst these, Eucalyptol is recognized for its anti-inflammatory and antimicrobial properties [11]. The results of this study indicate that the three selected Zingiberaceae herbs contain unique bioactive compounds, which contribute to their distinct pharmacological and biological activities. These differences make them suitable for use both as medicinal agents and as ingredients in various types of cosmetic products.



**Fig. 1** Gas Chromatography-Mass Spectrometry (GC-MS) spectrum of the *C. longa* rhizome.

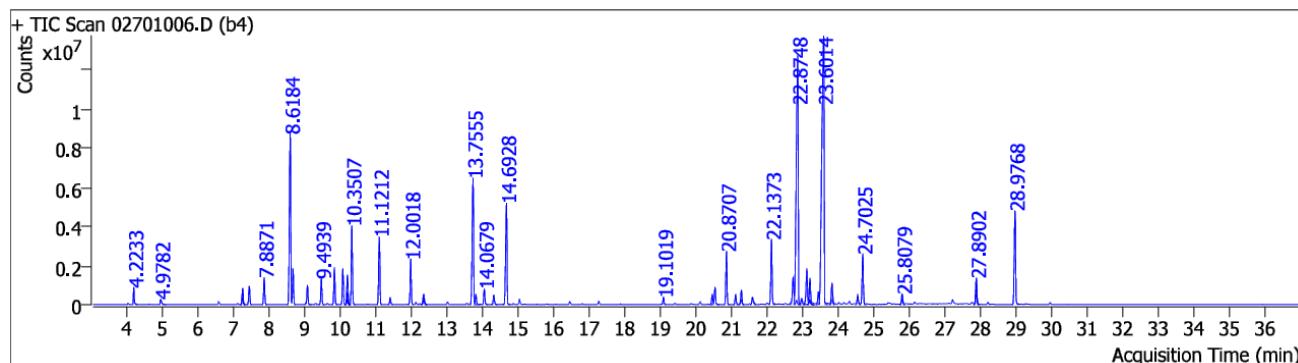
**Table 3** The list of compounds in *C. longa* rhizome Using GC-MS.

RT	Compound Name	Match Score	Area%-T	Area%-M
4.2427	2,3-Butanediol, [R-(R*,R*)]-	98.5	1.09	10.97
7.8857	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	88.7	0.99	9.94
7.8858	Camphene	97.9	1.11	11.16
8.6176	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	98.6	9.96	100.00
8.6966	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	98.0	1.48	14.83
9.4936	<i>alpha</i> -Phellandrene	97.9	1.59	15.99
9.8574	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	97.4	1.87	18.76
10.0978	<i>P</i> -Cymene	99.2	2.27	22.76
10.2245	Cyclohexane, 1-methylene-4-(1-methylethyl)-	96.8	1.84	18.51
10.3493	Eucalyptol	99.2	3.62	36.37
11.1202	<i>gamma</i> -Terpinene	99.3	3.63	36.49
10.0222	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	99.0	2.76	27.73
13.7354	(+)-2-Bornanone	99.3	2.46	24.69
14.7048	Terpinen-4-ol	98.1	9.78	98.25
20.8657	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene	97.0	1.07	10.70
21.2924	Caryophyllene	99.2	1.45	14.55
21.2925	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1 <i>R</i> -(1 <i>R</i> *,4 <i>Z</i> ,9 <i>S</i> *)]	78.7	1.12	11.28
22.1310	<i>cis-beta</i> -Farnesene	94.2	1.83	18.41
22.8358	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	97.2	8.89	89.25
23.1363	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	98.5	6.92	69.51
23.4505	<i>beta</i> -Bisabolene	96.5	1.42	14.22
23.5358	( <i>R</i> )-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	97.5	5.38	54.03
23.8440	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	98.0	7.85	78.88
27.1455	<i>aR</i> -Turmerone	98.2	5.35	53.72
27.2472	Turmerone	97.8	9.95	99.97
27.9543	2-Methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one	98.7	4.32	43.42

**Fig. 2** Gas Chromatography-Mass Spectrometry (GC-MS) spectrum of the *Z. montanum* rhizome.

**Table 4** The list of compounds in *Z. montanum* rhizome Using GC-MS.

RT	Compound Name	Match Score	Area%-T	Area%-M
4.2455	2,3-Butanediol, [R-(R*,R*)]-	98.7	2.13	16.85
7.8875	Camphene	98.2	1.54	12.23
8.6180	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	98.3	12.63	100.00
8.6969	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	97.7	1.96	15.48
9.4932	<i>alpha</i> -Phellandrene	97.9	1.51	11.99
10.3470	Eucalyptol	99.0	2.97	23.49
11.1193	<i>gamma</i> -Terpinene	99.4	4.28	33.89
11.9996	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	98.8	2.82	22.33
13.7372	(+)-2-Bornanone	99.3	4.01	31.78
14.0670	Isoborneol	99.2	1.61	12.76
14.7024	Terpinen-4-ol	97.9	10.66	84.42
20.5515	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1. <i>alpha</i> ,2. <i>beta</i> ,4. <i>beta</i> .)]	98.1	2.22	17.56
20.8647	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	96.4	1.27	10.09
21.2923	Caryophyllene	98.1	1.68	13.32
22.1477	Humulene	92.4	2.26	17.86
22.8377	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	95.6	10.49	83.08
23.1322	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	97.8	6.31	49.99
23.4490	<i>beta</i> -Bisabolene	94.6	2.17	17.16
23.5379	(R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	97.2	6.91	54.68
25.8118	Epicurzerenone	96.6	1.83	14.52
27.8859	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)-	98.6	1.95	15.45
28.3614	Neocurdione	98.2	1.93	15.25

**Fig. 3** Gas Chromatography-Mass Spectrometry (GC-MS) spectrum of the *C. aromatica* rhizome.**Table 5** The list of compounds in *C. aromatica* rhizome Using GC-MS.

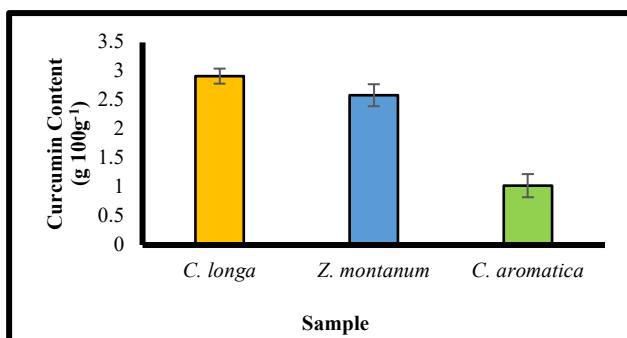
RT	Compound Name	Match Score	Area%-T	Area%-M
8.6184	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	98.3	10.98	38.64
10.3507	Eucalyptol	99.0	4.33	15.23
11.1212	<i>gamma</i> -Terpinene	99.4	3.71	13.04
13.7555	(+)-2-Bornanone	99.4	8.50	29.92
14.6928	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	97.7	6.62	23.28

**Table 5** (continued)

RT	Compound Name	Match Score	Area%‐T	Area%‐M
20.8707	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	96.6	3.01	10.57
22.1373	<i>cis</i> -beta-Farnesene	96.7	3.85	13.54
23.6014	(R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	97.0	28.42	100.00
24.7025	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	98.6	3.06	10.78
28.9768	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-	99.0	6.05	21.29

### Quantitative of curcumin in Zingiberaceae family

The curcumin content in three Zingiberaceae species was determined using a UV-Visible spectrophotometer at a wavelength of 427 nm. The results revealed that *C. longa* contained the highest amount of curcumin, followed by *Z. montanum*, while *C. aromaticata* had the lowest content, with values of  $2.92 \pm 0.13$ ,  $2.59 \pm 0.19$  and  $1.03 \pm 0.20$  g  $100\text{g}^{-1}$  fresh weight, respectively as shown in Fig. 4. The findings suggest that curcumin extracted from the Zingiberaceae family can be as an active ingredient in soap formulations. This study is consistent with previous research that analyzed the curcumin content in 25 different *C. longa* samples, in which the curcumin levels ranged from 1.60 to 3.44 %w/w [12]. It also aligns with another report that found the curcumin content in *Z. montanum* was 2.63 %w/w [13]. The results of this study reveal that the three species of Zingiberaceae contain varying levels of curcumin. These differences may be attributed to several factors, including plant species, cultivation areas, harvesting age and extraction methods. Curcumin is a compound with good antioxidant and anti-inflammatory properties that are beneficial for the skin. Therefore *C. longa* was selected as the main raw material for curcumin extraction, as it provides the highest amount of active compounds, thereby reducing production costs and enhancing product efficiency. Additionally, the variation in curcumin content among the three species provides essential baseline information for the development of other products utilizing curcumin as the active ingredient. These results confirm that the three herbs from the Zingiberaceae have unique active ingredients, which result in different pharmacological effects, both in traditional medicine and as ingredients in various types of cosmetics.

**Fig. 4** Quantity of curcumin in Zingiberaceae

### Quantitative of niacin in Broken Rice

The analysis of niacin content in sticky rice extracts from four cultivars cultivated in Ban Nam Yen, Dan Sai District, Loei Province, revealed that the highest amount was found in Daeng Mueang Loei, at  $5.69 \pm 0.19$  mg  $\text{kg}^{-1}$ . Whilst niacin was not detected (ND) in the Black sticky rice sample (Table 6), these results align with the research objective to evaluate niacin content in rice varieties for soap formulation development. The results indicate that the extract from Daeng Mueang Loei has high potential for development as an active ingredient in soap formulations, as it contains the highest amount of niacin. Niacinamide exhibits anti-inflammatory properties, enhances skin function, and protects the skin from free radicals. Additionally, the use of broken rice extract, a by-product of the rice milling industry, adds value to agricultural waste aligning with the concept of bio-economy and the efficient use of resources. The results demonstrate that the niacin content in broken rice is lower than previously reported, with earlier studies finding niacin levels in red and black rice ranging from 31.90 to 40.20 mg  $\text{kg}^{-1}$ . [14]. This difference likely reflects the use of whole rice grains in previous studies, as well as variations in extraction methods, storage conditions, or cultivation environments.

**Table 6** Niacin Content in Extracts from Four Sticky Rice Varieties

Sample	Niacin Content (mg $\text{kg}^{-1}$ )
Daeng Mueang Loei	$5.69 \pm 0.19$
Siw Kliang Mueang Loei	$4.44 \pm 0.03$
Black Sticky Rice	ND
Sticky Rice RD6	$4.21 \pm 0.08$

Note: ND (Not detected)

### Analytical method validation parameters

The accuracy of the method was verified according to the guidelines of the International Conference on Harmonization: ICH (2005), AOAC and International (2002). The results of the method validation study showed that all parameters met the specified criteria [15, 16]. The parameters including linearity, precision, accuracy, LOD (Limit of Detection), and LOQ (Limit of Quantification) were validated. Both methods were evaluated using standard substance graphs and showed good linear regression (correlation coefficients  $\geq 0.995$ ) within the concentration ranges of Blank-250 and 0.50-5.00 mg  $\text{L}^{-1}$ . For the analysis of the limits of detection (LOD) and limits of quantification

(LOQ) the values were 0.00, 0.01, 0.01, and 0.03 mg L<sup>-1</sup> for niacin and curcumin analysis, respectively. While, the precision analysis of the method showed that the Relative Standard Deviation (%RSD) values for both compounds were less than 11%, and the recovery studies for

both compounds showed values within the range of 80-110% as shown in Table 7. The analysis results demonstrate that the method used for analysis is accurate and suitable for the intended purpose of the analysis.

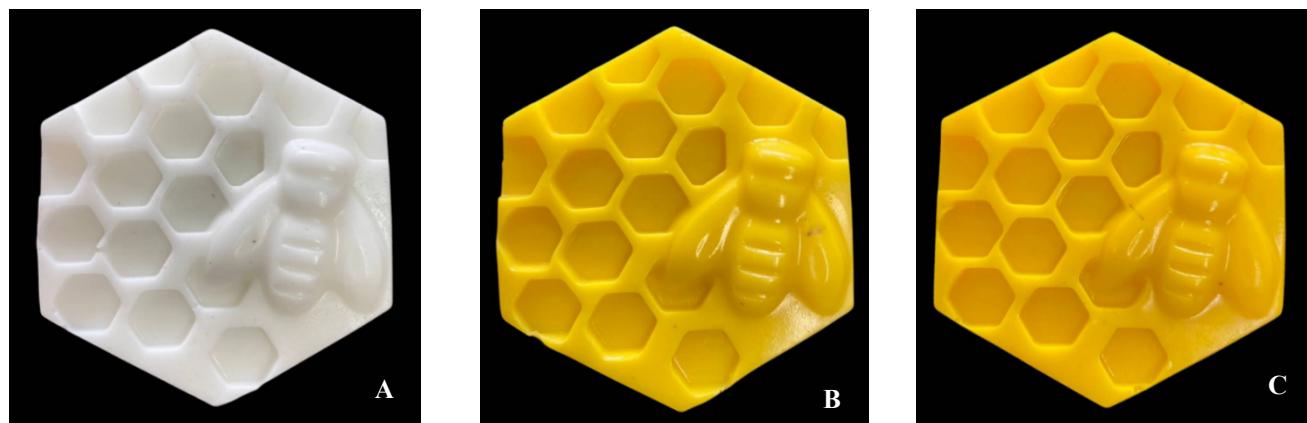
**Table 7** Method validation parameters for the quantification of curcumin and niacin.

parameters	Niacin Analysis	Curcumin Analysis
Linear Range (mg L <sup>-1</sup> )	Blank-250	0.50-5.00
Regression equation	$y = 15906x + 13893$	$y = 0.0219x - 0.001$
Correlation Coefficient (r <sup>2</sup> )	0.9985	0.9994
Recovery Percentage (% recovery)	98.17	96.05
Limit of Detection (LOD, mg L <sup>-1</sup> )	0.00	0.01
Limit of Quantification (LOQ, mg L <sup>-1</sup> )	0.01	0.03
Relative Standard Deviation (%RSD)	1.05	0.82

#### Physical and chemical properties of soap

The physical and chemical properties of soap in formulations 1 and 2 were tested and compared to the control formula across three parameters. The physical stability properties of both soap formulas were found to be of good quality compared to the control formula. The soaps had a smooth, fine texture without any air bubbles or cracks. The only differences between formulations were in color: the control formula was opaque white, formula 1 was yellow, and formula 2 exhibited a yellow-orange color. These color variations were due to the different ingredients used, indicating good physical stability and display desirable external characteristics for all formulas. The three soap formulas exhibited pH values between 9-10, consistent with standard soap formulations. Formula 1 exhibited

the highest alkalinity (pH 9.48), which may enhance cleaning effectiveness while potentially increasing the risk of skin irritation. In contrast, the control formula (pH 9.15) and formula 2 (pH 9.21) demonstrated slightly lower pH values, suggesting better chemical stability and skin compatibility. All formulas generated comparable foam quantities that remained stable for approximately 3 minutes. This demonstrates the good cleaning efficiency of each soap formula. Water solubility testing revealed formula 2 had the highest solubility (22% weight loss), followed by formula 1 (17%) and the control (15%). Formula 2 may have a shorter usage lifespan than the other formulas, but it may provide a softer and more moisturizing feel due to its good water solubility (Fig. 5). Despite these variations, all developed soaps met glycerin soap bar standards (CPS 665/2010) [17].



**Fig. 5** Soap Characteristics: A) Control formula B) Formula 1 and C) Formula 2

#### 4. Conclusion

The analysis of three Zingiberaceae species revealed distinct chemical compositions, each associated with specific bioactive properties. It was observed that *C. longa* and *Z. montanum* exhibited the highest concentrations of Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- (*cis*-sabinene). On the other hand,

*C. aromaticata* contained the highest concentration of (*R*)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene (*beta*-Curcumene). These bioactive compounds are known for their anti-inflammatory and antimicrobial activities. Furthermore, *C. longa* was found to possess the highest curcumin content, measured at  $2.92 \pm 0.13$  g 100 g<sup>-1</sup>. The analysis of niacin content in extracts from four rice

cultivars revealed that Daeng Mueang Loei contained the highest niacin level ( $5.69 \pm 0.19 \text{ mg kg}^{-1}$ ), whereas niacin was not detected in Black Sticky Rice (ND). The results of this study indicate that *C. longa* and Daeng Mueang Loei possess high potential as sources of curcumin and niacin, respectively. These findings suggest that both species are suitable for the development of specialized health and cosmetic products. The method validation results indicated that the analytical method demonstrated high precision and accuracy. The linearity values ( $R^2$ ) were  $\geq 0.995$ , precision (%RSD) was below 11%, and the analyte recoveries ranged from 80–110%. These results comply with the accepted standard criteria, confirming the reliability of the quantitative analytical method. During the development of soap formulations (control, formulation 1 and formulation 2), formulation 1 was found to exhibit superior stability in both physical and chemical properties relative to the control formulation. This formulation also demonstrated optimal texture, appearance, foaming ability, and water resistance, indicating its suitability as a candidate for further product development. This finding is in accordance with the Thai Community Product Standard for glycerin soap bars (CPS 665/2010).

## 5. Suggestions

1. Study the efficiency of the extraction process of niacin and curcumin to maximize the yield of active compounds.
2. Test the efficacy of the soap formulation in actual use with volunteers to evaluate its effects on skin condition and irritation.
3. Study other active compounds besides niacin and curcumin in the same raw material, such as phenolic compounds and vitamin E, to develop a more comprehensive formulation.

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## 7. Declaration of generative AI in scientific writing

AI techniques were utilised for enhancing readability and language quality.

## 8. CRediT author statement

**Napatsorn Wongpriaw** : Supervision, Investigation, Validation and Writing - Original Draft.

**Thitinan Thammasom** : Visualization.

**Bussabavadee Puttanu** : Project administration.

**Kitti Tanmuangpak** : Conceptualization.

**Wilailux Sudwilai** : Methodology, Data curation, Resources and Writing - Review & Editing

## 9. Research involving human and animals rights

Not applicable

## 10. Ethics Approval and Consent to Participate

Not applicable

## 11. Declaration of Competing Interest

The authors declare that there are no competing interests.

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