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Quantitative Phytochemical Analysis and Potential Applications of the Ethanol and Aqueous Ethanol Extracts of Some Selected Plant in Family *Zingiberaceae* Plants for Cosmeceutical and Health Promoting Food

Pannapa Powthong*

Department of Medical Technology, Rangsit University, Pathum Thani, Thailand

Corresponding author: Pannapa Powthong, Department of Medical Technology, Rangsit University, Pathum Thani, Thailand; Email: pannapa.p@rsu.ac.th

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Abstract

The rhizomes of the *Zingiberaceae* family are a vegetable widely used in many Asian nations, and their therapeutic properties have been acknowledged in many traditional recipes. In this work, 3 species of *Zingiberaceae* plants including *Curcuma longa*, *Curcuma zedoaria* and *Curcuma aromatic* were gathered and evaluated for their phytochemical contents, anti-inflammatory and anti-oxidant characteristics using the aqueous-ethanol (30:70%) and ethanol (95%) extraction and varying according to single and mixed extracts (1:1:1 and 2:1:1 ratio respectively) for determining the synergistic effects. The results indicated that extracts of the three selected plant contained at least 5 from 13 phytochemical constituents. The single aqueous-ethanol extract of *C. aromatica Salisb* and synergy achieved at 1:1:1 ratio of aqueous-ethanol extract showed the highest effective anti-inflammatory activity. The greatest antioxidant activity was found in a single ethanol extract of *C. zedoaria (Christm.)* and synergistically obtained at a 1:1:1 ratio of aqueous-ethanol extract. Furthermore, we discovered that combination extract produced greater outcomes than utilizing the mono extract alone. Our results demonstrate that screening for chosen *Zingiberaceae* plant extracts is a favorable representation of the value of screening for cosmetically and medicinal purposes.

Keywords: *Zingiberaceae*; *Curcuma longa*; *Curcuma zedoaria*; *Curcuma aromatica*

Introduction

Family *Zingiberaceae* has around 85 species of plants that are mostly grown in Asia, Central and South America, and Africa [25]. They recognized by their tuberous or non-tuberous rhizomes, which contain aromatic and therapeutic uses. Almost plants have been used by mankind as a source of food (spices and flavoring agents) and traditional medicine. Their rhizomes possess a number of medicinal, pharmacological, and nutritional properties like antiinflammatory, immunostimulatory, immunomodulatory and anticancer activity [12,21]. Although members of this genus have a similar appearance, their pharmacological and therapeutic activities range greatly [11].

Environmental stress can cause over production of oxidative stress; Reactive Oxygen Species (ROS), which is defined by cell/tissue injury and oxidative macro molecule damage. Oxidative stress can cause a number of diseases and accelerate aging, including atherosclerosis, diabetes, and cancer [23]. Despite the fact that the human body is created to have its own defense and repair systems to defend against oxidative damage, these systems are insufficient to completely avoid injury. Many reports have been indicating that many *Zingiberaceae* plants containing antioxidants and they may be useful in reducing oxidative damage [6]. The therapeutic effect and dose intake of natural extracts, on the other hand, vary owing to changes in the extraction method and active components included in it.

Our objective was to investigate the *in vitro* biological effect of the aqueous-ethanol and ethanol crude extract received from three medicinal plants in the family *Zingiberaceae* including turmeric (*Curcuma longa L.*), zedoary (*Curcuma zedoaria (Christm.)*), and wild turmeric (*Curcuma aromatica Salisb.*) on phytochemical constituent, anti-inflammatory, and antioxidant activity. The scientific result obtained here was possibility of developing this variety as an ingredient in health/medical cosmeceutical purposes or an alternative treatment to reduce anti-inflammatory drug side effects.

Materials and Methods

Plant material

Three medicinal plants in the family *Zingiberaceae* including turmeric, zedoary, and wild turmeric were purchased from the local market in Pathum Thani, Thailand. A taxonomist from drug and herbal product research and development center, College of Pharmacy, Rangsit University validated the botanical identity of each plant specimen. It was authenticated to be *Curcuma longa L.*, *Curcuma zedoaria (Christm.)*, and *Curcuma aromatica Salisb* belonging to family *Zingiberaceae*. Following that, the plant was washed, cut into little pieces, and dried at 40°C for 15-20 hrs. The dried material was crushed into powder and kept in an air

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tight plastic ba g at ro om temperature in a desiccator for subsequent examination.

Chemicals and reagents

Bovine serum albumin, Butylated Hydroxyl Toluene (BHT), trypsin, Tris-HCl, Thio Barbituric Acid (TBA), and Dimethyl Sulfoxide (DMSO) were obtained from Sigma-Aldrich Co., St. Louis, USA. All other basic reagents were of analytical grade.

Preparation of ethanolic crude extract

Ethanolic extraction was carried out for 5 days at 37°C using a sample of plant powders: 95% ethanol equivalent to 1:10 ratio. The solid has been removed by filtering it using Whatman No. 1 filter paper. For ethanol removal, the filtrate was processed through a rotary evaporator. Following that, each plant ethanolic crude extract was obtained. The crude was redissolved in Dimethyl Sulfoxide (DMSO) at a concentration of 1 g/mL and stored in a glass container at -20°C for subsequent analysis during the experiment.

The same technique was followed for aqueous-ethanol extraction with 30% distilled water: 70% ethanol.

Preliminary Phytochemical Screening of plant crude extract

Each plant crude extract was subjected to the following assays to assess the presence or absence of fundamental phytochemicals: anthraquinones, tannins, saponins, flavonoids, glycosides, cardiac glycosides, terpenoids, steroids, alkaloids, coumarins, phenols, protein amino acids, fat and oil using standard biochemical procedures [9,20].

Anti-in lammatory activity of plant extract

Inhibition of albumin denaturation: The anti-inflammatory activity of each plant in family *Zingiberaceae* was studied by using inhibition of albumin denaturation technique which was studied according to previous study [18,26] followed with minor modifications. The reaction mixture was consisting of 100 µl of test extracts samples at different concentrations (50–5,000 µg/mL) and 500 µl of 1% aqueous solution of bovine albumin fraction. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured at 660 nm. The Positive and negative control was acetyl salicylic acid and distilled water respectively. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

% inhibition = x 100

Where A0 is the absorbance of the control (control contained 1% aqueous solution of bovine albumin and distill water), and A1 is the absorbance of the sample.

Inhibition of protein denaturation method using egg albumin: The anti-inflammatory effect of each plant in the *Zingiberaceae* family was investigated using the inhibition of egg albumin denaturation approach described before [32,2], with minor changes. The reaction mixture contained 200 µl of test extracts at various concentrations (50–5,000 µg/mL), 20 µl of eggs albumin (from hen egg), and 280 µl of phosphate buffered saline (PBS, pH 6.4). The sample extracts were incubated at 37°C for 15 min and then heated to 70°C for 15 min, after cooling the samples the turbidity was measured at 660 nm. Acetyl salicylic acid and distilled water were used as positive and negative controls, respectively. The experiment was carried out three times. The following percentage inhibition of protein denaturation was calculated:

% inhibition = x 100

Where A0 is the absorbance of the negative control, and A1 is the absorbance of the sample.

In-vitro proteinase inhibitory assay

The *in-vitro* proteinase inhibitory was performed as described previously [19]. Briefly, the test extract at various concentrations (50–5,000 µg/mL) was prepared. Then 500 of each concentration were mixed with 500 µL of 0.06 mg of trypsin in 20 mM Tris HCl buffer (pH 7.4). The mixture was incubated at 37^0C for 5 min and 500 µL of 0.8% (W/V) casein was added. The mixture was re-incubated at 37^0C for another 20 min, then 1000 µL of 10% glacial acetic acid was added to stop the reaction. The mixture tube was then centrifuged at 3000 rpm for 10 min. The resulting supernatant was subjected to measure the absorbance at a wavelength of 210 nm. The experiment was performed triplicated. The following percentage of inhibition proteinase inhibitory activity was calculated:

% inhibition = x 100

Where A0 is the absorbance of the negative control (distilled water), and A1 is the absorbance of the sample.

Anti-oxidant assay of plant extract

Lipid Peroxidase (LPO): Malondilaldehyde (MDA), the last product of lipid oxidation, was measured with Thiobarbituric Acid Reactive Substance (TBARS) method as modified from previous study [22]. Briefly, 50–5,000 µg/mL of plant extract dilution were prepared with water as a solvent. 50 µl of this solution was mixed by 50 µl of egg yolk 10% w/v KCl solution, 300 µl of 20%w/v acetic acid (pH 3.5), and 300 µl of Thiobarbituric Acid (TBA) solution. The mixture then was boiled at 95^0C for 1 hrs. Then 750 µl of butanol was adding and leave it until its color as changed into pink. The mixture then centrifuged at 3,000 g at 250C for 10 min. Supernatant absorbance measured at 532 nm. Alpha tocopherol and distilled water were used as positive and negative controls, respectively. The experiment was carried out three times. Lipid peroxidation inhibition percentage was calculated by the following formula:

% inhibition = x 100

Where A0 is the absorbance of the negative control (distilled water), and A1 is the absorbance of the sample.

Metal chelating complex assay

The ferrous ion chelating activity of methanol and aqueous-ethanol extracts was estimated by the method [15]. Briefly 100 µL of the extracts samples at different concentrations

(50–5,000 µg/mL) were added to a solution of 2 mM $FeCl_2$ (10 µL). The reaction was initiated by the addition 20 µL of ferrozine (5 mM) and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then measured at 562 nm. Ascorbic acid was used as a positive control. The experiment was performed in triplicate. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the following formula:

%Ferrous ions chelating activity = x 100

Where A0 is the absorbance of the control (control contained FeCl2 and ferrozine; complex formation molecules), and A1 is the absorbance of the sample.

Hydrogen Peroxide Scavenging Activity (HPSA)

In the present study, the ability of plant extracts to scavenge hydrogen peroxide can be modified according to the previous report [8]. Briefly, 200 µl of various concentrations of plant extract concentration (50–5,000 µg/mL) in methanol was mixed with 100 µl ml of a solution of hydrogen peroxide (20 mM) in phosphate buffer (50 mM pH 7.4). The mixture was left to stand for 10 min and the absorbance was then measured at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. The assay was carried out in triplicate. Alpha tocopherol was use as a positive control. The percentage of hydrogen peroxide scavenging is calculated as follows:

% Scavenged (H_2O_2) = [(Ai – At) / Ai] × 100

Where Ai is the absorbance of control and at is the absorbance of test.

Statistical Analysis

All the experiments were performed and studied in triplicate. Results represent the mean ± standard deviation.

Student t-test was performed to test the significance of differences between means at the 5% level using the SPSS ver.22 software (Chicago, IL, USA) to conduct the statistical analysis.

Results

Phytochemical analysis

As shown in Table 1, the aqueous-ethanol and ethanol crude extract received from three medicinal plants in the family *Zingiberaceae* were found to possess various phytochemicals or polyphenols such as flavonoids, phenolics, steroids, glycosides, sapronins, coumarins, protein/ amino acids, and fat/oil, etc.

Furthermore, it was discovered that each solvent allowed the identification of the same phytochemical except for protein and amino acids and saponins in the aqueous-ethanol crude extract of *C. longa L* and *C. zedoaria (Christm.)*, but not in ethanol solvent.

Table 1: Phytochemical result of plant crude extract.

Phytochemicals	**Aqueous-ethanol crude extract**			**Ethanol crude extract**		
	Curcuma longa L.	*Curcuma zedoaria (Christm.)*	*Curcuma aromatic*	*Curcuma longa L.*	*Curcuma zedoaria (Christm.)*	*Curcuma aromatic*
Anthraquinones	-	-	-	-	-	-
Tannins	-	-	-	-	-	-
Saponins	-	+	-	-	-	-
Flavonoids	+	+	+	+	+	+
Glycosides	-	+	+	-	+	+
Cardiac glycosides	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-
Steroids	-	+	+	-	+	+
Alkaloids	-	-	-	-	-	-

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Coumarins	+	-	-	+	-	-
Phenols	+	+	+	+	+	+
Protein & Amino acids	+	-	-	-	-	-
Fat & Oil	+	+	+	+	+	+

- = absent; + = present

Inhibition of albumin denaturation

Protein denaturation is a well-known cause of inflammation. The capacity of the plant extract to prevent protein denaturation was investigated as part of the study into the mechanism of anti-inflammation action. It was denoted that heat-induced albumin denaturation was inhibited by *Zingiberaceae* plants (Figure 1 and Table 2). Within a concentration range of 50 to 5,000 g/mL, the percentage of inhibition was measured to be 30.27-93.42 %. Maximum inhibition was observed from aqueous-ethanolextract of *C. aromatica Salisb.* with an IC_{50} value of 0.61 µg/mL, follow by ethanol extract of *C. zedoaria (Christm.)* and aqueous-ethanol extract of *C. zedoaria (Christm.)*(IC_{50} value 2.79 and 4.81 µg/ml respectively). The 1:1:1 ratio of aqueous-ethanol demonstrated the strongest activity (IC 50 value 3.50 µg/ml). The t-test analysis showed that there is significant difference in the level of inhibition compared to acetyl salicylic acid, a standard anti-inflammation drug which showed the maximum inhibition 93.03% at the concentration of 5,000 µg/mL (IC_{50} value 26.88 µg/mL).

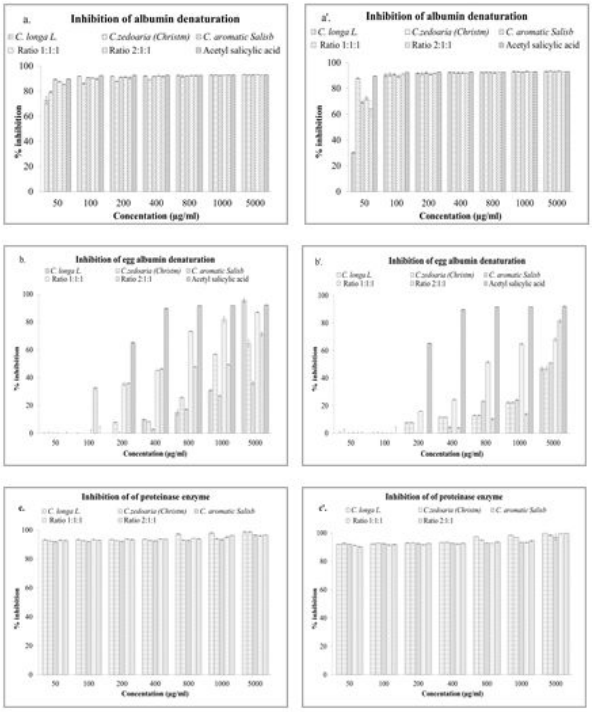

Figure 1: Anti-inflammatory assays of different concentrations of aqueous-ethanol and Ethanol extracts of *Curcuma longa L.*, *Curcuma zedoaria (Christm.)*, and *Curcuma aromatica Salisb.* (a) The inhibition of protein denaturation of aqueous-ethanol extract determined in various concentrations varying from 50 -5000 µg/mL; (a') The inhibition of protein denaturation of ethanol extract was evaluated in concentration varying from 50-5000 µg/mL.A gradual increase in scavenging potential of the extract was obtained with an increase in concentration; (b) The inhibition of protein denaturation method using egg albumin of aqueous-ethanol extract; (b') The inhibition of protein denaturation method using egg albumin of ethanol extract. A gradual increase in scavenging potential of the extract was obtained with an increase in concentration (c) The inhibition of proteinase activity of aqueous-ethanol extract was determined; (c') The inhibition of proteinase activity of ethanol extract was determined. A gradual increase in scavenging potential of the extract was obtained with an increase in concentration. n=3 (mean ± standard deviation).

Table 2: IC 50 Values for anti-inflammation activities by plant extracts and standard.

Extract/Positive control IC 50	Values for albumin denaturation inhibition ± SEM (µg /mL)	Values for egg albumin denaturation inhibition ± SEM (µg /mL)	Values for Proteinase inhibition ± SEM (µg /mL)
Aqueous-ethanol extract *C. longa L.*	35.65 ± 1.98	1626.23 ± 62.86	649.24 ± 17.41
Aqueous-ethanol extract *C. zedoaria (Christm.)*	4.81 ± 0.75	842.44 ± 29.75	401.96 ± 9.20
Aqueous-ethanol extract *C. aromatica Salisb*	0.61 ± 0.02	810.12 ± 19.96	400.97 ± 7.55
Aqueous-ethanol extract ratio 1:1:1	3.50 ± 0.57	292.09 ± 83.91	399.66 ± 2.67
Aqueous-ethanol extract ratio 2:1:1	5.79 ± 0.81	9.01 ± 0.25	867.58 ± 51.26
Ethanol extract *C. longa L.*	36.51 ± 4.33	2840.93 ± 25.72	675.69 ± 44.15
Ethanol extract *C. zedoaria (Christm.)*	2.79 ± 0.63	2840.93 ± 25.65	872.94 ± 29.15
Ethanol extract *C. aromatica Salisb*	26.29 ± 4.10	971.90 ± 95.12	398.92 ± 1.65
Ethanol extract ratio 1:1:1	13.81 ± 3.16	480.59 ± 83.78	404.40 ± 63.85
Ethanol extract ratio 2:1:1	36.51 ± 4.86	4394.16 ± 22.46	404.81 ± 5.07
(Standard)	26.88 ± 1.24	180.15 ± 9.57	-

Data are given as Mean ± SEM (n=3).

Inhibition of egg albumin denaturation

The anti-inflammatory properties of *Zingiberaceae* plants were tested *in vitro* against denaturation of egg albumin. The present findings exhibited a concentration dependent inhibition of egg protein (albumin) denaturation by most of extract. The percentage of inhibition was found to be 4.29-81.61%. The ethanol extract of C. has the maximum level of proteinase inhibitory action. Aqueous-ethanol extract *C. aromatica Salisb.* has the maximum level of albumin denaturation inhibition with an IC 50 value of 810.12 µg/mL, follow by aqueous-ethanol extract of *C. zedoaria (Christm.)* and ethanol extract of *C. aromatica Salisb.* (IC_{50} value 842.44 and 971.90 µg/ml respectively). The 2:1:1 ratio of aqueous-ethanol demonstrated the strongest activity (IC_{50} value 9.01 µg/ml). The t-test analysis showed that there is significant difference in the level of inhibition compared with the standard (Acetyl salicylic acid) it was 65.24-92.20% (IC_{50} value 180.15 µg/ml) throughout the concentration range of 50 to 5,000 µg/ml. As shown in Figure 1 and Table 2.

Proteinase inhibitory activity

Proteinases have been linked to arthritic symptoms. Neutrophil lysosomal granules are known to be a rich source of proteinase. All extracts from the *Zingiberaceae* family demonstrated substantial anti-proteinase activity in a concentration-dependent manner. The majority of them showed more than 90% inhibition at the lowest concentration as 50 µg/ml. The percentage of inhibition was found to be 90.18-100%. The highest value of proteinase inhibitory activity was detected in ethanol extract of *C. aromatica Salisb.* with an IC 50 value of 398.92 µg/mL, follow by aqueous-ethanol extract of *C. aromatica Salisb.* and aqueous-ethanolextract of *C. zedoaria (Christm.)* with an IC_{50} value of 400.97 and 401.96 µg/mL respectively. The 1:1:1 ratio of aqueous-ethanol demonstrated the strongest activity (IC_{50} value 399.66 µg/ml) as shown in Figure 1 and Table 2.

Anti-lipid-peroxidation with reactive substance (TBARS) method

From the Figure 2 and Table 3, it was showed that all extracts of the fascinating plants in the *Zingiberaceae* family were capable to inhibit egg yolk cholesterol peroxidation reaction in a concentration-dependent manner compared to the blank. The inhibition percentage was determined to be 6.45-54.81 %. The highest value of anti-lipid peroxidation which performed by ethanol extract of *C. zedoaria (Christm.)* with an IC_{50} value of 3.64 µg/mL, follow by aqueous-ethanol extract of *C. zedoaria (Christm.)* and ethanol extract of *C. longa L.* with an IC_{50} value of 10.54 and 119.60µg/mL respectively. The 1:1:1 ratio of aqueous

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-ethanoldemonstrated the strongest activity (IC_{50} value 2.37 µg/ml). The t-test analysis revealed a significant difference in the amount of inhibition when compared to standard alpha-tocopherol (IC_{50} 448.56 µg/mL). As shown in Figure 2 and Table 3.

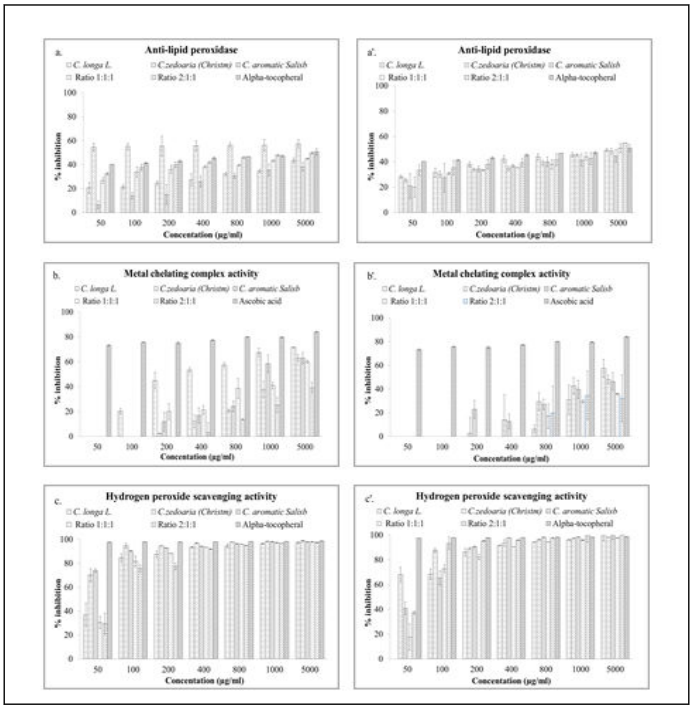

Figure 2: Anti-oxidant assays of different concentrations of aqueous-ethanol and Ethanol extracts of *Curcuma longa L., Curcuma zedoaria (Christm.), and Curcuma aromatica Salisb.* a) Anti-lipid peroxidase activity of aqueous-ethanol extract determined in various concentrations varying from 50-5000 µg/mL; (a') Anti-lipid peroxidase activity of ethanol extract was evaluated in concentration varying from 50 -5000 µg/mL. A gradual increase in scavenging potential of the extract was obtained with an increase in concentration (b) Metal chelating complex activity of aqueous-ethanol extract; (b') Metal chelating complex activity of ethanol extract. A gradual increase in scavenging potential of the extract was obtained with an increase in concentration (c) Hydrogen peroxide scavenging activity of aqueous-ethanol extract was determined; (c') Hydrogen peroxide scavenging activity of ethanol extract was determined.

A gradual increase in scavenging potential of the extract was obtained with an increase in concentration. n=3 (mean ± standard deviation).

Table 3: IC 50 values for hydrogen peroxide scavenging activity by plant extracts and standard.

Extract/Positive control IC 50	Values for lipid peroxidase inhibition ± SEM (µg /mL)	Values for metal chelating complex activity ± SEM (µg /mL)	Values for hydrogen peroxide scavenging activity ± SEM (µg /mL)
Aqueous-ethanol extract *C. longa L.*	863.48 ± 96.6	127.17 ± 9.21	19.13 ± 3.46
Aqueous-ethanol extract *C. zedoaria (Christm.)*	10.54 ± 0.64	975.54 ± 15.34	34.20 ± 2.96
Aqueous-ethanol extract *C. aromatica Salisb*	317.07 ± 9.63	685.34 ± 5.83	6.93 ± 2.25
Aqueous-ethanol extract ratio 1:1:1	2.37 ± 0. 76	504.73 ± 2.46	18.75 ± 1.40
Aqueous-ethanol extract ratio 2:1:1	90.88 ± 2.46	902.64 ± 23.53	7.33 ± 1.59
Ethanol extract *C. longa L.*	119.60 ± 1.45	984.05 ± 0.22	88.06 ± 7.88
Ethanol extract *C. zedoaria (Christm.)*	387.78 ± 3.75	597.45 ± 6.84	5.09 ± 1.35
Ethanol extract *C. aromatica Salisb*	3.64 ± 2.92	422.89 ± 5.89	101.47 ± 3.20
Ethanol extract ratio 1:1:1	11.33 ± 1.07	809.06 ± 1.54	8.05 ± 1.24

Ethanol extract ratio 2:1:1	496.56 ± 8.30	793.98 ± 2.02	36.32 ± 3.92
Standard	448.56 ± 11.98	405.13 ± 6.97	398.74 ± 1.27

Data are given as Mean ± SEM (n=3).

Ferrous ions chelating activity

All extracts of the interesting plants in the family *Zingiberaceae* were able to chelate ferrous ions in a concentration-dependent manner. Across the whole concentration range of 50-5,000 g/ml, the inhibition percentage was estimated to be 2.56-71.69%. At the lowest concentration as 400 µg/mL, aqueous-ethanol extract of *C. longa L.* exerted a strongest chelating effect (53.31%) with an IC_{50} value of 127.17 µg/mL followed by ethanol extract of *C. aromatica Salisb.* and aqueous-ethanol extract of *C. aromatica Salisb.* (IC_{50} value of 422.89 and 685.34 µg/ml respectively). The 1:1:1 ratio of aqueous-ethanol reveled the highest activity (IC 50 value 504.73 µg/ml). This activity was similar to that obtained with the standard chelator ascorbic acid (IC_{50} 448.56 µg/mL). As shown in figure 2 and table 3.

Hydrogen Peroxide Scavenging Activity (HPSA)

The aqueous-ethanol and ethanol crude extract were screened for H_2O_2 radical scavenging activity in which the level of inhibition was calculated to be 17.52-100%. The highest activity was detected in ethanol extract of *C. zedoaria (Christm.)* at the lowest concentration as 50 µg/mL with an IC 50 value 5.09 µg/ml) followed by aqueous-ethanol extract of *C. aromatica Salisb.* and *C. longa L.* (IC 50 value 6.93 and 19.13 µg/ml respectively). The 2:1:1 ratio of aqueous-ethanol showed the highest activity (IC_{50} value 7.33 µg/ml). The t-test analysis showed that there is significant difference in the H_2O_2 radical scavenging activity among the different extract of test sample and standard alpha tocopherol with an IC_{50} value 398.74 µg/ml. As shown in figure 2 and table 3.

Discussion

The results of preliminary quantitative phytochemical screening of aqueous-ethanol and ethanol extracts revealed the presence of multiple polar and non-polar chemical constituents. Flavonoids, phenolics, steroids, glycosides, sapronins, coumarins, protein/ amino acids, and fat/oil were present in the extracts. Aqueous-ethanol extraction allowed some phytochemical present than in ethanol extraction. It was correlated with previous study report that many essential oils are found in *Zingiberaceae* plants, including terpenes, alcohols, ketones, flavonoids, carotenoids, and phytoestrogens which can be extracted using organic solvents [13,16,29,31]. *Curcuminoids*, which include curcumin, demethoxycurcumin, and bisdemethoxycurcumin, have been identified as the major active components [24]. Several studies have discovered that plant extracts high in polyphenols and important phyto-constituents such as alkaloids, glycosides, terpenoids, saponin, phenols, and steroids exhibit significant antioxidant and free radical scavenging activities in a variety of antioxidant models [10,3].

Although numerous procedures for assessing the anti-oxidant activity of biological samples have been developed, it is extremely difficult to analyze each antioxidant component independently since each antioxidant molecule is difficult to isolate from organic extracts. As a result, we aimed to confirm the biological efficacy in different *in vitro* anti-oxidant models by use crude extract in this study.

Protein denaturation and proteinases in arthritic responses have been implicated as a source of inflammation. The capacity of the aqueous-ethanol and ethanol extracts to prevent protein denaturation both bovine serum and egg albumin were evaluated as part of the experiment into the mechanism of anti-inflammation action. Currently, the single aqueous-ethanol extract of *C. aromatica Salisb.* and synergy achieved at 1:1:1 ratio of aqueous-ethanol extract exhibited the effectively prevented heat-induced protein denaturation and proteinases inhibition comparing to Acetyl salicylic acid, a standard anti-inflammation drug at the concentration of 50-5000 µg/ml.

Previous studies report that curcumin has antiinflammatory e ffects[4,28], and one of its mechanisms is most likely mediated by its capacity to inhibit Cyclooxygenase-2 (COX-2), Lipoxygenase (LOX), and Inducible Nitric Oxide Synthase (INOS) (iNOS) which are key enzymes that mediate inflammatory processes [27,17]. Oral curcumin has been demonstrated in animal experiments to produce antinociceptive effects [30], with the involvement of ATP-sensitive potassium channels [7]. Curcumin has showed potential in pilot human trials for relieving symptoms of rheumatoid arthritis and inflammatory bowel illness [14].

Oxidative stress enhanced several diseases, including cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases. Antioxidant properties in the extract applied in this study demonstrated effective *in vitro* findings, including anti-lipid-peroxidation, ferrous ions chelating activity, and hydrogen peroxide scavenging. The single ethanol extract of *C. zedoaria (Christm*.) and synergy obtained at a 1:1:1 aqueous-ethanol extract ratio effectively prevented harmful reactive oxygen species. The antioxidant activity of plants is mostly acquired from active molecules or major phytoconstituents that they possess [33]. Indeed, studies have revealed that curcumin, the primary active component, may rapidly scavenge free radicals, mainly to its H-atom donation from the phenolic group. Furthermore, it is associated in the scavenging of these radicals in peroxidation processes and is a possible antioxidant against the production of hydrogen peroxide and superoxide radicals [34,1,5].

Regarding the extraction methods used the combination between water and ethanol and ethanol alone were used to obtain good and effective results. It was demonstrated that aqueous-ethanol extraction method and synergistic effects

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among the combinations of selected plants in this group on the anti-inflammation and anti-oxidant was documented.

Conclusion

The above results give a conclusion that the rhizome of a selected plant in the Zingiberaceae family contains polyherbal formulations with varying concentrations that have anti-inflammation and anti-oxidant properties valuable enough to warrant development as a safe cosmeceutical or functional food product for anti-aging and to reduce inflammatory disease. Furthermore, this study suggests that combining the selected plants in this group may be utilized as a novel method, allowing us to employ a lower concentration of the extract or active components, lowering the possibility of harmful consequences.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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