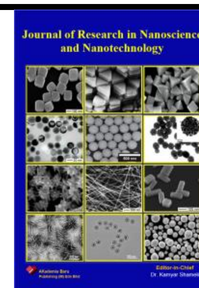
Journal of Research in Nanoscience and Nanotechnology

Journal homepage:
http://akademiabaru.com/submit/index.php/jrnn/index
ISSN: 2773-6180



Comparison of Chemical Composition and Antioxidant Activity of Extracted *Zingiberaceae* Rhizome using Subcritical Water Extraction

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https://doi.org/10.37934/jrnn.8.1.1322

ABSTRACT

In Malaysia, many local herbs can take advantage of their benefit for health products, cosmetics, and food production. In this research, fresh *Zingiberacea* rhizomes including *Curcuma Longa*, *Curcuma Zedoaria*, *Curcuma Xanthorrhiza*, *Zingiber Officinale*, and *Zingiber Zerumbet* rhizomes were extracted using the subcritical water extraction (SWE) unit located at AM Zaideen Ventures Sdn Bhd in Kuala Lumpur (Malaysia). The focus of this research is to analyze the freeze-dried powder extracts from the fresh *Zingiberaceae* rhizome for the phytochemical screening and bioactive compounds. Operating parameter used in the extraction process was fixed at temperature of 110°C, pressure at 10 bar and extraction time of 15 minutes. The freeze-dried powder extracts were screened for its phytochemicals inclusive alkaloids, steroids, terpenoids, glycosides, tannin and saponin. For bioactive compounds analysis, extracts were evaluated for Total Phenolic Content, Total Flavonoid Content, DPPH scavenging activity assay, and bioactive compound using Gas-Chromatography Mass Spectrophotometry analysis. The results show that there was a different presence of phytochemicals compounds in different *Zingiberaceae* extracts. At 10mg/ml concentration of powder extract, *Zingiberaceae* rhizomes showed variations in total phenol and flavonoid content, ranging from 134.65 to 285.19 mg GAE/g and from 22.58 to 181.62 mg QE/g, respectively. For DPPH radical scavenging activity, observed at 10mg/ml of freeze-dried extract, all *Zingiberacea* demonstrated significance antioxidant activity of more than 85% inhibition. Bioactive compounds detected in *Zingiberaceae* rhizomes shows significance quality. This finding supports the exploration of indigenous herbs market in Malaysia and the potential of water-based extracts on fresh *Zingiberaceae* rhizomes.

Keywords:
Zingiberacea rhizomes, subcritical water extraction, DPPH

Received: 4 Apr. 2023 Revised: 8 Jun. 2023 Accepted: 25 Jul. 2023 Published: 1 Sep. 2023

1. Introduction

Subcritical Water Extraction (SWE) is a relatively new method that uses only water and a brief extraction period to extract less-polar molecules. Subcritical water is kept liquid at high pressure between 10 and 20 MPa and temperature between 100 and 374°C [1]. Subcritical water is a sufficient method for creating pharmacologically active plant extracts and developing new nutritional supplements and cosmetic items because of its exceptional qualities [2]. Subcritical water, which is affordable, secure, and effective, takes the role of conventional organic solvents in this method. Subcritical water is a sufficient method for creating pharmacologically active plant extracts and developing new nutritional supplements and cosmetic items because of its exceptional qualities [2]. The fact that subcritical water's polarity can be drastically reduced with rising temperature is a special and practical property of the substance. As a result, subcritical water may act like methanol or ethanol. Subcritical water, which is affordable, secure, and effective, takes the role of conventional organic solvents in this method. Subcritical water is a sufficient method for creating pharmacologically active plant extracts and developing new nutritional supplements and cosmetic items because of its exceptional qualities [2].

Zingiberaceae family is a well-researched group of plants with tremendous potential as a nutraceutical and therapeutic product for improving human health. It is commonly be grown in Asia particularly Malaysia, Indonesia, Thailand, China and India [3-4]. In Malaysia, common name for *Zingibereace* was dissimilar based on the species: *Zingiber zerumbet* known as bitter ginger and lempoyang; *Zingiber officinale* recognized as ginger or halia, *Curcuma longa* or turmeric were named in Malay as *Kunyit*, *Curcuma xanthorrhiza* known as *Temulawak*, and *Curcuma zedoaria* was locally be known as *Kunyit Putih.* These *Zingiberacae* were used extensively in variety of culinary, dishes and drinks [3-4]. It was shown that the ginger family's rhizomes have relatively diverse scent compositions, resulting in chemicals with sweet-citrus, spicy, and camphorous properties. It was known that the essential oils with specific aromatic characteristics are present in all parts of *Zingiberaceae* plants, but they are most abundant in the rhizomes and leaves. Additionally, it concluded that certain *Zingiberaceae* plants utilized in food may exhibit certain characteristics, such as the presence of a particular component in some types of rhizomes but not in others [5].

Besides, the *Zingiberaceae* rhizome were used in traditional medicine since ancient time and known for their diverse range of phytochemicals, which have various pharmacological, biological, and dietary advantages. For instance, *Zingiber zerumbet* had a potential health benefit as it has bioactive compound which is zerumbone, a natural compound found in the essential oil, and it belongs to a group of organic compounds called sesquiterpenes and possess beneficial properties such as reducing inflammation, alleviating allergic reactions, and acting as an antioxidant [6]. *Zingiber officinale* has several chemicals responsible for its medicinal properties, such as anti-arthritis, anti-inflammatory, antidiabetic, antibacterial, antifungal and anticancer [7-8]. The combination of zingerone, shogaols, gingerols, and volatile oils gives the ginger root its distinctive flavor and aroma, with sulfur-containing chemicals (allicin, alliin, and ajoene) and enzymes (allinase, peroxidase, and myrosinase) in its rhizome [8]. Curcumin and curcuminoids is the name of the primary polyphenol present in the rhizome of *Curcuma* family. Hepatoprotective, anti-inflammatory, antibacterial, antioxidant, and anticancer capabilities are only a few of the pharmacological effects it possesses in *Curcuma longa* [9]. The rhizome oil in the *Curcuma zedoaria* contains the antioxidants curzerene and epicurzerene. It was said to have potent anti-inflammatory, anticancer, and antioxidant properties [10]. These bioactive compounds play a vital role in disease prevention by acting as powerful

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antioxidants that scavenge free radicals, interrupt radical chain reactions, and chelate metals in the body [11].

Phytochemicals, which are present in large quantities in fruits, vegetables and herbs may provide defense against free radical damage. Plants were knowns to have beneficial phytochemicals that may serve as a natural complement to the body's requirement for antioxidants. The quantity of antioxidants in many plants has been shown in several research. For instance, vitamins A, C, and E, as well as phenolic substances produced from plants such as flavonoids, tannins, and lignins, all serve as antioxidants [12]. This practically states that a high concentration of phytochemicals in the extracts has a wide range of benefits for humans which act as an antioxidant. Therefore, in this research, fresh *Zingiberacea* rhizome includes *Zingiber zerumbet*, *Zingiber officinale*, *Curcuma longa*, *Curcuma xanthorrhiza* and *Curcuma zedoaria* were utilized in subcritical water extraction process to explore the benefits of water-based local herbs for its phytochemical screening and bioactive compounds.

2. Materials and Methods

2.1 Chemicals

All chemicals and reagent used were of analytical reagent grade. The apparatus and chemicals used for the analysis are conducted at the Halal Laboratory which is located at the Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia (UTM).

2.2 Raw Materials

The selected *Zingiberaceae* rhizome collected from Kizaherbs in Temerloh, Pahang were as shown in Figure 1. This includes *Curcuma longa*, *Curcuma xanthorrhiza*, *Curcuma zedoaria*, *Zingiber officinale*, and *Zingiber zerumbet* rhizome.

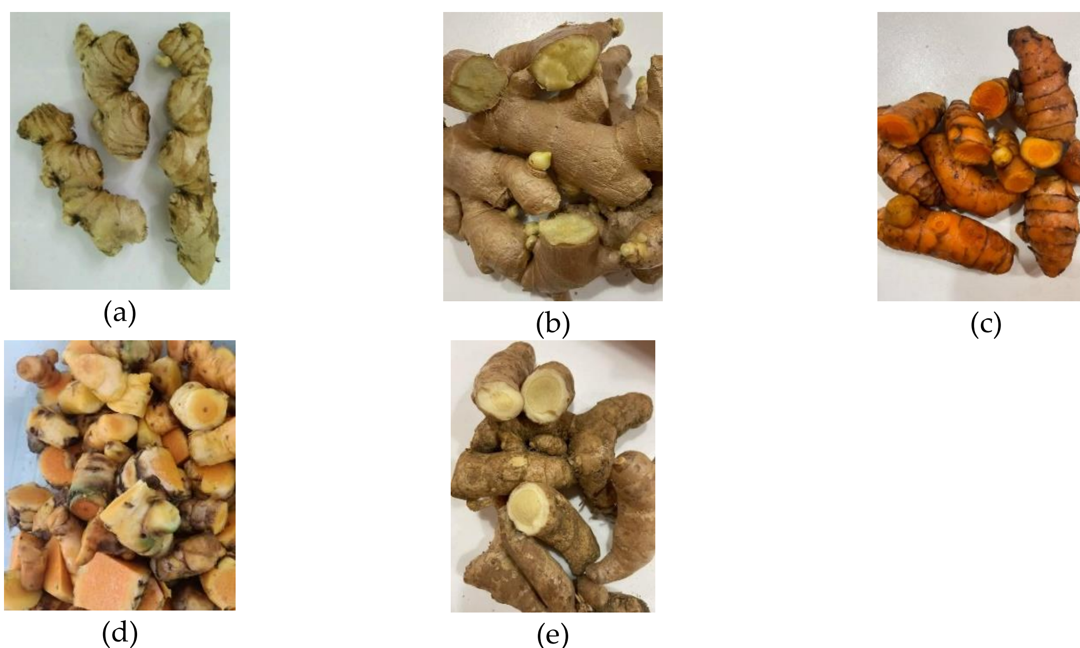

Fig. 1. Rhizome for selected *Zingiberaceae* (a) *Zingiber zerumbet*, (b) *Zingiber Officinale*, (c) *Curcuma longa*, (d) *Curcuma xanthorrhirza*, (e) *Curcuma zedoaria*

2.3 Sample Preparation

To eliminate dirt and impurities, the raw material, was first washed with filtered water and coarse salt. Sample were cut and blended to smaller particle size to increase surface area.

2.4 Subcritical Water Extraction

The Subcritical Water Extraction (SWE) equipment from AM Zaideen Ventures Sdn. Bhd. was used to carry out the SWE procedure. A fixed 1:3 ratio was set between the source material and the solvent. Inserting food-grade nitrogen gas into the reactor was allowed to control the initial pressure. Extraction process were conducted at 10 bar, 15 minutes of extraction time and process temperature at 110°C. The crude extracted product was transferred from extraction vessel to the cooling chamber after the extraction phase was finished in order to shorten the time of sample was exposed to high temperatures. Samples were filtered and the liquid extract were collected in food grade container. Sample were stored at -20°C in freezer (Berjaya, 1000 L) prior to freeze drying process

2.5 Freeze Drying

The liquid extracted samples underwent 60 L capacity freeze dryer (CUDDON FD80, New Zealand) located at Incubator HALAL, UTM Pagoh after the extraction process [13]. Liquid extracts were spread on the trays and placed onto a single product chamber of a pilot scale freeze dryer. The drying operation was carried out in three stages, which are freezing, primary drying, and secondary drying. The refrigeration conditions were designed as follows: freezing temperature of –20°C; sublimation temperature of –20°C to 0°C; and vacuum level of 0.34 mbar. The isothermal desorption temperature ranged from 0°C to 60°C in a vacuum of 0.060 mbar. The freeze-drying process took approximately 34 hours until the final product reached a temperature of 40°C. The extracts produced in powder form were then analyzed for their phytochemical screening and bioactive compound.

2.6 Phytochemical Compound Screening

Qualitative screening for phytochemicals of the selected *Zingiberaceae* rhizome on the presence of alkaloids, steroids, terpenoids, tannins, saponins, and glycosides was detected by conducting several methods.

2.6.1 Alkaloid

The presence of alkaloids in the freeze dried extract was analyzed by using Wagner's test [14]. About 1 g of Potassium iodide and 0.635 g iodine were dissolved in 2.5 ml distilled water and the solution was diluted with 50 ml of distilled water in preparing Wagner's reagent. Then, a crude extract solution, 0.015 g extract was stirred with 6 ml of 1% Hydrochloric Acid in the water bath for 5 min was prepared. The analyzed presence of alkaloids in the extract was tested by added 2 to 3 drops of Wagner's reagent into the crude extract solution that had been filtered. A brown-coloured precipitate indicated the presence of alkaloids.

2.6.2 Steroid and terpenoid

The presence of steroid and terpenoid was identified by using Salkowski test [14]. About 0.1 g of extract in a sample bottle was prepared. Then 2ml Chloroform was added into the extract and shake the solution. Next, 2 ml of sulphuric acid was added to the shaken solution. The appearance of reddish-brown colour at the lower layer indicates the presence of steroids and the yellow colour shows the presence of terpenoids

2.6.3 Taniin

Next, the presence of tannin was analyzed by adding a few drops of 5% Ferric Chloride into the extract solution (0.5 g extract into a sample bottle and add 10 ml of distilled water) and observing black or blue-green coloration of the precipitate which indicates the presence of tannins [14].

2.6.4 Saponin

The presence of saponin was observing by adding 0.5 g of extracts into 10 ml of distilled water. Then, the solution was vortexed for a few seconds and the solution was remained stay in the water bath for 5 min. If the formation of frothing shows, there is the presence of saponins [14].

2.6.5 Glycoside

The presence of glycoside was analyzed by adding a few drops of 10% aqueous sodium hydroxide to the extract solution (1mg/ml). A yellowish colour appeared indicates the presence of glycosides [15].

2.7 Bioactive Compund

The bioactive compound of the selected *Zingiberaceae* rhizome were analyze inclusive of Total Phenolic Content, Total Flavonoid Content, Antioxidant Activity, and screening of bioactive compound using Gas-Chromatography Mass Spectrometry (GCMS).

2.7.1 Total phenolic content

The Total Phenolic Content (TPC) was conducted using the Folin-Ciocalteu method with some modifications [16]. About 0.5 ml of powder extract in water prepared at 10mg/ml was mixed with 2.5 ml of Folin-Ciocalteu reagent in distilled water (1:10 v/v). The mixture was then vortexed and was rest for 5 minutes. Then, 2 ml of 7.5% (w/v) of sodium carbonate was added to the mixture and incubated for about 2 hours. The absorbance was measured using UV-Vis Spectrometry at 750nm wavelength. The TPC of each crude extract was determined from the standard curve of gallic acid range from 1 to 1000 ppm and the results was expressed as mg gallic acid equivalent (GAE) per gram of extract.

2.7.2 Total flavonoid content

The analysis of Total Flavonoid Content (TFC) was conducted using the Aluminium Colorimetric method with some modifications [17]. About 2 ml of powder extract in water at 10mg/ml was mixed with 3 ml of 5% Aluminium Chloride. The mixture was vortexed and incubated for about 30 minutes. The absorbance was measured using UV-Vis Spectrometry at 437 nm. The Blank use was methanol. The TFC the crude extract was determined from the standard curve of quercetin range from 1 to 1000 ppm and the results were expressed as mg quercetin equivalent per gram of extract (mg QE/g).

2.7.3 Antioxidant activity

Antioxidant activity was analyzed by using the DPPH assays with some modifications [18]. The powder extract was initially diluted in methanol at 10mg/ml and was sonicated alternate with vortex for 10 minutes. A total of 2 ml of 0.1 mM DPPH methanolic solution was mixed with 2 ml of each diluted extract. The solution was forcefully shaken with a vortex and was incubated for 30 minutes at room temperature in a dark place. A UV-Visible Spectrophotometer with a cuvette was used to test the sample's absorbance at 517 nm. The antioxidant activity was calculated using Eq. (1) where A is the absorbance of the control (methanolic DPPH solution without crude extract) and B is the absorbance of the sample (methanolic DPPH solution in the presence of extract).

$$Antioxidant\ activity\ (\%) = \frac{(A - B)}{A} \times 100 \quad (1)$$

2.7.4 Identification of bioactive compound

Bioactive compound of each extract was screened using Gas Chromatography-Mass Spectrometry (GC-MS) Test [19]. 1 μl of extract diluted in Hexane (1mg/ml) was directly injected into the GC model HP 6890 with HP 5973 mass selective detector (Agilent, Wilmington, USA) and Chemstation data system was used to identify the chemicals. At an electron energy of 70 eV, a source temperature of 250°C, and a scan rate of 0.81 scans per second, electron impact-mass of the extracted component was conducted. A 5%-phenyl methylpolysiloxane capillary column with a length of 30 m and a thickness of 0.25 mm and 0.25 μm (HP-5MS, Agilent 19091S-433, Wilmington, USA) was utilised. The oven temperature was set constant at 70°C for 2 min and programmed to rise up to 230°C at a rate of 7°C/min and then mained at same temperature for 6 min. Helium at a flow rate of 1ml/min was used as a carrier gas. The splitless injector was kept at 250°C. The injector and MS detector temperature gas 250°C and 280°C. Lastly, compounds detected were compared with the data in library NIST17 for determination of bioactive compounds.

3. Results and Discussion

The result for each analysis that have be done which was phytochemical analysis for presence of alkaloid, saponin, tannin, glycosides, steroid and terpenoid. While, the results for bioactive compound analysis were including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA).

3.1 Phytochemical Compound Analysis

Table 1 depicts the phytochemical compound that is present in the selected *Zingiberaceae* rhizome. The results show that the selected *Zingiberaceae* rhizomes were found to contain various phytochemical compounds. All selected *Zingiberaceae* rhizome extracts contained alkaloids, tannins, and saponins, although steroids were only found in *Zingiber zerumbet*, *Curcuma xanthorrhiza*, *Curcuma longa*, and Zingiber officinale. While terpenoids were only found in *Zingiber zerumbet* and *Curcuma xanthorrhiza*. *Zingiber zerumbet* exhibited the presence of all phytochemical compounds.

Table 1. Comparison of phytochemical screening of *Zingiberace* rhizome water-based extracts

	Curcuma zedoaria	*Zingiber zerumbet*	*Curcuma xanthorrhiza*	*Curcuma longa*	*Zingiber officinale*
Alkaloid*	+++	++	++	+	+++
Saponin*	+++	++	+	+	+
Steroid**	-	+	+	+	+
Terpenoid**	-	+	+	-	-
Tannin**	+	+	+	+	+
Glycoside**s	-	+	-	+	-

Indicator : * + low concentration, ++ medium concentration,+++ high Concentration, ** + present, - absent

The low concentration of alkaloids in turmeric may be attributed to the specific genetic makeup and biosynthesis pathways of the plant, which result in the predominant accumulation of curcuminoids rather than alkaloids [20]. *Curcuma zedoaria* had the highest concentration of saponin and follow by *Zingiber zerumbet*. The high presence of saponins in *Curcuma zedoaria* is due to the plant's high concentration of triterpenoid saponins. Tannins are a class of polyphenols abundantly present in various plant species such as *Zingiber officinale*, *Curcuma longa*, *Curcuma zedoaria*, *Zingiber zerumbet*, and *Curcuma xanthorrhiza*. These compounds contribute to the bitter taste experienced when consuming these spices, while also exhibiting numerous health-promoting properties. The wide range of phytochemicals in plants can contribute to different health benefits, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [21]. However, the effectiveness and safety of these compounds depend on factors such as their concentration, availability, interactions in the body, and individual variations in metabolism.

3.2 Bioactive Compund Analysis

Table 2 shows the comparison of bioactive compound in the selected *Zingiberaceae* rhizomes. The results displayed variations in total phenolic content, flavonoid content, antioxidant activity and bioactive compound. The crude extracts from the five *Zingiberaceae* plants contained very high amounts of phenolic compounds which resulted in stabilizing free radicals due to their being strong electron donor [22]. *Zingiber zerumbet* rhizome had the highest total phenolic content with 285.185 ± 0.787 mg GAE/g freeze-dried extract, while *Curcuma zedoaria* had the lowest with 134.65 ± 0.77 mg GAE/g. the significant total phenolic content in *Zingiber zerumbet* and *Curcuma longa* may contribute to their traditional use as medicinal herbs in Southeast Asia [24].

Table 2. Bioactive compound analysis

Rhizome	Total Phenolic (mg GAE/g)	Total Flavonoid (mg QE/g)	Antioxidant Activity (%)	Major Bioactive compounds
Curcuma xanthorrhiza	218.340 ± 1.40	144.70 ± 0.12	88.30 ± 0.28	Curcumene, β-Curcumene
Zingiber officinale	207.86 ± 0.23	22.58 ± 0.22	89.82 ± 0.26	Zingerone, Phenol, Tumerone
Curcuma longa	277.24 ± 0.48	181.62 ± 0.04	89.34 ± 0.13	Glycol, Tumerone
Zingiber zerumbet	285.19 ± 0.787	57.31 ± 0.28	87.33 ± 0.23	Oleyl Chloride, 1-(2-Butoxyethoxy) ethanol, Phenol, Zerumbone
Curcuma zedoaria	134.65 ± 0.77	43.53 ± 0.27	86.97 ± 0.28	9,17-Octadecadienal, Phenol, Glycol, Curzerenone

Meanwhile, *Curcuma longa* exhibited the highest flavonoid content with 181.62 ± 0.04 mg QE/g. This study is similar with recent study by [23] that shows high amount of total flavonoid content in *Curcume longa* while lowest amount of total flavonoid content found in *Zingiber officinale.* This study shows that *Curcuma longa* and *Curcuma xanthorrhiza* contain the highest amount of flavonoids compared to other species of the rhizomes. The high flavonoid content in *Curcuma longa* and *Curcuma xanthorrhiza* may due to the presence of curcuminoid [24].

For antioxidant activity observed at 10mg/ml of freeze-dried extract, all *Zingiberacea* demonstrated significance antioxidant activity of more than 85% inhibition. The exceptional antioxidant activity of *Curcuma xanthorrhiza* can be attributed to the presence of several compounds with antioxidant properties, including curcuminoids, xanthorrhizol, and demethoxycurcumin. These compounds have been proven to effectively neutralize free radicals and hinder the oxidation of lipids [24].

Different *Zingiberaceae* rhizome has a different amount of bioactive compounds which lead to different nature of benefits. *Zingiber zerumbet* exhibits high phenolic content, potent antioxidant activity, and contains bioactive compounds like zerumbone as shown in the result, suggesting its potential therapeutic benefits. *Zingiber zerumbet*, with its high phenolic content, potent antioxidant activity, and bioactive compounds like zerumbone, holds promise as a natural source of therapeutic compounds for promoting human health.

4. Conclusions

The study aimed to identify the benefits of the selected *Zingiberaceae* by analyzing the phytochemical and bioactive compounds. The selected *Zingiberaceae* rhizomes contain various phytochemical compounds such as alkaloids, tannins, saponins, steroids, terpenoids, and glycosides, with *Zingiber zerumbet* exhibiting the presence of all phytochemical compounds, suggesting their potential health benefits. The comparison of bioactive compounds in the selected *Zingiberaceae* rhizomes indicates that *Zingiber zerumbet*, with its high phenolic content, antioxidant activity, and bioactive compounds like zerumbone, shows potential as a valuable natural source of therapeutic compounds for promoting human health. Thus, this research has the potential for future development in a new market for products made from *Zingiberaceae* rhizomes that has a lot of benefit for human health.

Acknowledgement

The authors would like to thanks AM Zaideen Ventures Sdn. Bhd. and Malaysia-Japan International Institute of Technology, UTM Kuala Lumpur, for the facilities and research allocation throughout research activities.

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