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Research article

Effect of Extraction Solvents on Antioxidant and Antibacterial Activity of *Zingiber montanum* Rhizomes

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Abstract: The *Zingiber montanum* rhizome has been utilized for its antiviral, immunomodulatory, anti-inflammatory, and antibacterial properties for a long time, particularly in Malaysia, Indonesia, and Thailand. Additionally, the rhizome has been a traditional ingredient in Asian cosmetic products. This study aimed to investigate the impact of different extracting solvents (hexane, dichloromethane, acetone, ethanol, methanol, 50% ethanol, and 75% ethanol) on the phenolic content, as well as the antioxidant and antibacterial activities of *Zingiber montanum*. The antioxidant activity was evaluated using two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric-reducing antioxidant power (FRAP) assay, while antibacterial activity was tested against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* strains. The methanol extract demonstrated the highest phenolic content, while the ethanol extract exhibited a slightly lower amount. In the DPPH assay, the methanol extract showed an IC_{50} value of 36.89 ± 2.53 μg/mL, whereas the ethanol extract displayed a marginally higher value of 38.89 ± 0.27 μg/mL. In terms of ferric-reducing antioxidant power, the ethanol extract had slightly higher FRAP values (78.65 ± 4.73 mg AAE/g) than the methanol extract (76.09 ± 4.57 mg AAE/g). All extracts exhibited low activity against the three tested bacterial strains. Ethanol extract demonstrated the most antibacterial activity, with a clear zone ranging from 10.50 to 12.00 mm. The results suggest that ethanol is a suitable solvent for extracting *Zingiber montanum* rhizome for value-added materials application for cosmetic products.

Keywords: *Zingiber montanum*, solvent extraction, antioxidant, antibacterial

1. Introduction

Zingiber montanum (J. Koenig) Link ex A. Dietr., also known as Phlai in Thai, is a biennial plant that belongs to the *Zingiber* genus, which comprises around 85 species. Its rhizomes possess a bitter taste and have been traditionally used for culinary, dietary supplement purposes, and medicinal such as relieving dysentery, asthma, bruises, constipation, dyspepsia, gastritis, stomach bloating, and stomach-ache [1]. *Z. montanum* has shown potential for further development due to its wide range of pharmacological properties, such as anti-inflammatory, antibacterial,

antifungal, antioxidant, antihistaminic, anticholinesterase, and smooth muscle relaxant activities [2]. These properties make it an attractive area for further research and development, with the potential for new treatments for various medical conditions and cosmetics applications.

Z. montanum is frequently utilized as an ingredient in cosmetic products [3] available in the market, as the active compounds found in the plant can help address various skin concerns such as reducing acne, moisturizing the skin, minimizing wrinkles, alleviating skin allergies, and enhancing skin radiance. Phytochemical investigation of *Z. montanum* rhizomes revealed the presence of numerous bioactive compounds such as alkaloids, flavonoids, terpenoids, saponins, tannins, phlorotannins, steroids, and glycosides [4]. The important process in isolating active compounds is extraction, which can be influenced by different factors such as chemical composition, extraction technique, sample particle size, duration, and solvent. The solid-liquid extraction method, utilizing various solvents, is commonly employed for extracting active compounds from plants [5]. The ethanol extract of *Z. montanum* exhibited antibacterial properties and antioxidant activity in several studies [6,7]. The complex curcuminoids, cassumunins A, B, and C, isolated from acetone extract of *Z. montanum* rhizomes, exhibited more potent antioxidant and anti-inflammatory properties than curcumin [8]. Methanol extract from *Z. montanum* displayed DPPH radical scavenging activity with a half maximal inhibitory concentration (IC_{50}) value of 0.34 mg/mL [9]. The volatile oils of some *Zingiber* plants displayed multiple biological activities, including antioxidant effects [10]. Indeed, numerous studies have been conducted on extracting active ingredients using a single solvent or a mixture of solvents. The choice of solvent used for extraction can significantly impact the types and quantities of active components extracted [11]. Thus, using different solvents for extraction can yield different sets of active ingredients and, consequently, affect the overall efficacy of the resulting extract. Researchers must consider this when selecting a solvent or a combination of solvents for their extraction process.

Z. montanum is a plant that is found throughout all regions of Thailand. The DPPH antioxidant activity of *Z. montanum* ethanol extracts from various areas in Thailand revealed that the levels of antioxidants present in the plant varied significantly depending on the cultivation area. The percentage of antioxidants was 57.63-80.88, and the sample from the North showed the highest amount of antioxidants [12]. As a result, the researcher aims to explore the use of solvent extraction to obtain substantial amounts of antioxidants and antibacterial agents, as well as analyze the constituents of the extract acquired from southern Thailand. The total phenolic content will be analyzed as a quality control measure to ensure the products used in the production cycle are of high quality. Additionally, the study will assess the antioxidation and antibacterial activity of the extracts, providing essential information for effectively utilizing *Z. montanum* as a cosmetic ingredient.

2. Materials and Methods

2.1 Plant materials

Fresh *Zingiber montanum* rhizomes were purchased from a local market in Phattalung province, Thailand, in November 2022. Dr. Paveena Kaewubon, a plant taxonomist at the Department of Biology, Thaksin University, Thailand, identified the rhizomes.

2.2 Chemicals and reagents

Gallic acid was purchased from Sigma-Aldrich Chemicals. Folin-Ciolcateu reagent was obtained from Fisher Scientific. Fluka Chemie GmbH supplied 2,2-Diphenyl-1-picrylhydrazyl (DPPH), while ascorbic acid and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were acquired from Merck. All other reagents used in this study were of analytical grade. Mueller Hinton Agar was obtained from Hi-media, and Nutrient Agar was purchased from Difco.

2.3 Preparation of crude extracts

The fresh rhizomes of *Z. montanum* were washed with tap water to remove dirt, sliced into small pieces, and then dried in an oven at 50°C for three days. The dried rhizomes were ground into coarse powder by a grinding machine. 100 g of the powdered samples were soaked in 300 mL of seven different solvents (hexane, dichloromethane, acetone, ethanol, methanol, 50% ethanol, and 75% ethanol) at room temperature for thirty minutes and then subjected to ultrasound-assisted extraction at 45 KHz (35°C) for thirty minutes. The solutions were filtered through a Whatman filter No.1. The extracted solutions were evaporated below

40°C using a rotary evaporator and then freeze-dried. Seven brown extracts were obtained and stored at 4°C until further investigation.

2.4 Determination of total phenolic content

The total phenolic content of *Z. montanum* extracts was determined using the Folin-Ciocalteu colorimetric method, based on the procedure described by Iqbal and coworkers [13], with some modifications. In brief, 0.2 mL of each extract solution (10 mg/mL) was mixed with 1.0 mL of 10% Folin-Ciocalteu reagent. After 5 min, 0.8 mL of 20% Na_2CO_3 solution was added. The solution was vortexed for 15s and left in the dark for 90 min at 25°C for color development. The absorbance was measured using a UV–Vis spectrophotometer (SHIMADZU UV-1700, USA) at 725 nm against a blank. The experiment was repeated three times at each concentration, and the total phenolic contents were calculated based on the calibration curve of gallic acid (0.01-0.07 mg/mL). The results were expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g extract).

2.5 DPPH radical scavenging assay

The DPPH radical scavenging activity of *Z. montanum* extracts was evaluated using the method of Vichit and Saewan (2015) [14] with slight modifications. The DPPH assay measures the electron transfer of antioxidants towards the stable DPPH radical at 517 nm. In brief, a reaction mixture containing 0.2 mL of the sample and 1.8 mL of 0.1 mM DPPH solution was vortexed ultimately and incubated for 30 min in the dark at room temperature. A UV-Vis spectrophotometer was used to measure the absorbance at 517 nm, and each sample was tested in triplicate using ascorbic acid as a positive standard. The scavenging activity was calculated as the percentage of inhibition and derived using the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

$A_{control}$ is the absorbance of DPPH radical with ethanol (without extract), and A_{sample} is the absorbance in the presence of the DPPH radical and test sample. The scavenging activity of *Z. montanum* extract was expressed as the IC_{50} value, which represents the concentration (mg/mL) of the extract required to scavenge 50% of the DPPH radical.

2.6 Ferric reducing antioxidant power (FRAP) assay

The ferric-reducing antioxidant power (FRAP) was determined according to the method described by Benzie and Strain (1996) [15] with some modifications. Briefly, 0.2 mL of the sample at a concentration of 0.5 mg/mL was mixed with 1.8 mL of the FRAP reagent and incubated at room temperature for 5 min in the dark. The absorbance was measured at 593 nm using a UV-Vis spectrophotometer. Samples were measured in triplicates, and ascorbic acid was used as the standard. The results were expressed in milligrams equivalent of ascorbic acid per gram of dry extract (mg AAE/g extract).

2.7 Antibacterial testing

An agar well diffusion assay was performed with some modifications based on the method described by Shimanuki and Knox (2000) [16] to evaluate the antibacterial activity of *Z. montanum* extracts against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The cell cultures were initially grown on nutrient agar (NA) medium and incubated for 24 h at 37 °C. The final turbidity of the cultures was adjusted to a 0.5 Mcfarland standard (1.5×10^8 CFU/mL) using 0.85% w/v NaCl. The Mueller Hinton agar (MHA) surface was inoculated by spreading a volume of the microbial inoculum over it. A sterile cork borer was then used to aseptically punch a hole with a diameter of 6 mm in the agar plate surface. In each well, 60 µL of the test sample (0.1 mg/mL) was added using a sterile pipette. The plates were then incubated at 37°C for 24 h. After the incubation period, the diameters of the inhibition zones were measured in mm. The experiment was conducted in triplicate, with ampicillin as a positive control for *S. aureus* and ciprofloxacin as a positive control for *E. coli* and *P. aeruginosa*. 10% DMSO was used as the negative control.

2.8 Gas chromatography-Mass spectrometry (GC-MS) analysis

The ethanol extract of *Z. montanum* was analyzed using a gas chromatography-mass spectrometry (GC-MS) system equipped with an Agilent 19091S-433UI GC and a mass spectrometer, which was fitted with an HP-5ms capillary column (5% phenyl methyl polysiloxane, 30 m length, 250 µm diameter, 0.25 µm film

thickness, and temperature range of -60 to 350°C). The GC-MS system was also interfaced with a flame ionization detector (FID). In a gas chromatography analysis, helium was used as the carrier gas with a flow rate of 3.0 mL/min in split mode with a ratio of 20:1. A 1 µL sample was injected into the column through an injector with a temperature set to 250°C. The column temperature was initially set at 50°C for 5 min, then gradually increased at a rate of 5°C /min until reaching 250°C without any holding period. The temperature was then held at 280°C for 3 min at a 5°C/min program rate. The total elution time was 49 min. The relative percent amount of each component was calculated by comparing its average peak area to the total area. The ion source temperature was maintained at 230°C. The MS Spectrum was obtained using electron ionization at 70 eV. After separating the column, the components were identified and further analyzed by FID in scan mode from 50-1000 amu. The total running time was 650 min. To determine the compounds, the spectrum of the unknown compound was compared to known compounds in the NIST MS 1.4 structural library, which allowed for the determination of the compound names, molecular weights, and structures.

2.9 Statistical analysis

The experiments were conducted in triplicate. Values were reported as the mean ± standard deviation (SD). ANOVA tests were performed using GenStat software to determine significant differences in extracts. Differences with a probability (p) value of ≤ 0.05, indicating a 95% confidence level, were considered statistically significant. Simple linear regression analysis was used in the data analysis.

3. Results and Discussion

3.1 Percentage yield

The rhizomes of *Z. montanum* were extracted by maceration with various solvents, including hexane, dichloromethane, acetone, ethanol, methanol, 75% ethanol, and 50% ethanol, at a ratio of 1:3 w/v for 3 h, followed by ultrasound-assisted extraction for 30 min. After removing the solvent, seven different extracts were obtained. The percentage yield for each extract is shown in Table 1. The results demonstrated that 50% ethanol and methanol were the most effective solvents, producing the highest weight of crude extracts, with yields of 5.78% and 5.37%, respectively. The substantial mass of the polar solvent extract implies that it predominantly contains the polar components found in *Z. montanum*. However, the percentage yield in this study was lower than that reported by Rungruang and coworkers [17], who achieved a yield of 5.57% by extraction with 50% ethanol using an orbital shaker at 150 rpm for 24 h. This difference in yield may be due to the shorter extraction time used in this study with an ultrasonic bath. The results suggested that the primary constituents in the rhizomes of *Z. montanum* were likely highly polar.

Table 1. The percentage yield of crude extract and total phenolic content of *Z. montanum* extract

Extract[1]	% Yield of crude extract	Total phenolic content (mg GAE/g extract)
PH	2.30	24.83 ± 0.31^{f}
PD	3.98	53.74 ± 0.84^{d}
PA	3.73	57.53 ± 2.92^{c}
PE	3.72	62.22 ± 0.30^{b}
PM	5.37	71.45 ± 1.45^{a}
PE75	2.15	50.30 ± 0.91^{e}
PE50	5.78	49.52 ± 0.32^{e}

[1] PH: hexane extract, PD: dichloromethane extract, PA: acetone extract, PE: ethanol extract, PM: methanol extract, PE75: 75% ethanol extract, and PE50: 50% ethanol extract

3.2 Total phenolic content

Phenolic compounds are a diverse group of secondary metabolites produced by plants, and their concentrations and compositions depend on various factors such as plant species, growth conditions, and extraction techniques. Phenolics are polar substances reported to possess various beneficial properties, including antioxidant, anti-inflammatory, and anticancer activities. Therefore, measuring the total phenolic content of a plant extract can serve as an initial screening method for antioxidant activity. The total phenolic

content of *Z. montanum* extracts was determined using the Folin-Ciocalteu reagent. The reagent is reduced in the presence of phenolic compounds, producing a blue-colored complex. The intensity of the blue color is directly proportional to the number of phenolic compounds in the extract [18].

The total phenolic content of *Z. montanum* extracts was expressed as milligram equivalents of gallic acid per gram of extract (mg GAE/g extract), which was calculated using the gallic acid standard curve $y = 11.441x + 0.0146$ ($R^2 = 0.9985$), as shown in Table 1. The total phenolic content ranged from 24.83 ± 0.31 to 71.45 ± 1.45 mg GAE/g extract, with the methanol extract exhibiting the highest phenolic content while the hexane extract showed the lowest. A polar solvent can extract a broader range of phenolic compounds, both polar and nonpolar, leading to a higher total phenolic content. Suggests that methanol is a suitable solvent for phenolic extraction from *Z. montanum*, while higher mixes of polar solvents like 50% ethanol and 75% ethanol are not ideal for this study. These results are different from those of previous studies that reported an ethanol-water mixture (40–60% v/v) as being more effective for extracting phenolic compounds from plants than a mono-solvent system [19-21], due to the wide range of phenols that the ethanol-water mixtures can dissolve [22]. Additionally, a study by Rungruang and coworkers, which used 50% ethanol as a solvent, found a phenolic content value of 213.16 mg/g extract [17], which differs from this study's result of 49.52 ± 0.32 mg GAE/g extract. These discrepancies may be due to differences in plant ages and extraction processes.

3.3 DPPH radical scavenging activity

The ability of *Z. montanum* extracts to scavenge free radicals was evaluated using the DPPH radical scavenging method. The reaction involves substances that donate radical hydrogen species, which convert the DPPH radical to its non-radical form, DPPH-H. A significant reduction of DPPH indicates a large amount of hydrogen radical in the reaction. The results revealed that the methanol extract exhibited the highest DPPH radical scavenging activity, followed by ethanol extract and acetone extract, with IC_{50} values of 36.89 ± 2.53, 38.89 ± 0.27, and 40.63 ± 1.23 μg/mL, respectively. The results were consistent with the total phenolic content of the extract, as the high range of phenolic compounds in the methanol extract resulted in a significant decrease in the DPPH content. The extracts showed good antioxidant efficiency. However, the standard ascorbic acid still exhibited a lower IC_{50} value of 22.82 ± 0.20 μg/mL, as shown in Table 2.

Table 2. The antioxidant activity of *Z. montanum* extract

Extract[1]	DPPH radical scavenging activity (IC_{50}, μg/mL)	Ferric-reducing antioxidant power activity (FRAP value, mg AAE/g extract)
PH	264.34 ± 0.51^{f}	48.85 ± 4.57^{e}
PD	68.36 ± 0.15^{d}	73.57 ± 0.78^{b}
PA	40.63 ± 1.23^{b}	73.81 ± 3.23^{b}
PE	38.89 ± 0.27^{a,b}	76.09 ± 4.57^{a,b}
PM	36.89 ± 2.53^{a}	78.65 ± 4.73^{a}
PE75	64.70 ± 3.23^{c}	67.51 ± 1.89^{c}
PE50	151.14 ± 3.12^{e}	62.87 ± 0.11^{d}
ascorbic acid	22.82 ± 0.20	-

[1] PH: hexane extract, PD: dichloromethane extract, PA: acetone extract, PE: ethanol extract, PM: methanol extract, PE75: 75% ethanol extract, and PE50: 50% ethanol extract

Our study found a significant difference in IC_{50} values between the ethanol extract (38.89 μg/mL) and that reported by Rungruang and coworkers in 2021 (213.16 μg/mL). However, our results revealed that the efficacy of the ethanol extract was inferior to that of ascorbic acid by a factor of 1.7. Likewise, Rungruang and coworkers found the extract less effective than ascorbic acid by a factor of 1.2. The difference in the scavenging activity of the ethanol extract and the other report could be attributed to the age of the plant and the drying technique employed [17]. Specifically, using a freeze-drying approach in our study may have resulted in a lower residual solvent or water content than the method used by Rungruang coworkers in 2021. Nonetheless, it is worth noting that this drying process may potentially lead to the loss of some antioxidants. Cassumunins A-C are the phenolic antioxidants in *Z. montanum*, as previously reported by Masuda and Jitoe in 1994 [8] and Nagano and coworkers in 1997 [23].

3.4 Ferric-reducing antioxidant power (FRAP) activity

Ferric-reducing antioxidant power activity of the extract involved the transfer of a single electron with an antioxidant compound. The activity of the extract was reported as FRAP value in mg AAE/g extract. The high FRAP value corresponds to the good reducing ability of the extract. The study of *Z. montanum* extracts on reducing ferric iron was conducted at a 0.5 mg/mL concentration. The FRAP values were calculated from the ascorbic acid calibration curve: y= 0.0254x – 0.0504 (R^2 = 0.9935), and the result is shown in Table 2. Although the polarity of methanol was close to 75% ethanol, the phenolic content and antioxidant properties differed. The ethanol and methanol extracts had relatively the most effective in reducing ferric ions with FRAP values of 78.65 ± 4.73 and 76.09 ± 4.57 mg AAE/g extract, respectively. The activity trend was slightly different from the DPPH assay, but the ethanol and methanol extract values were not significantly different.

The results of both methods indicated that methanol and ethanol extracts were similar constituents. They also showed higher antioxidant activity than the other *Z. montanum* extracts. The results confirmed that extraction with different solvents led to various constituents and activities. In addition, the antioxidant activity of crude extracts was inversely proportional to the phenolic content. An extract that contains a large amount of total phenolic compounds also showed good antioxidant activity, while the extract with low total phenolic content displayed poor antioxidant activity. Although the ethanol and methanol extracts had very similar antioxidant activity, in terms of safety and utilization, ethanol is the most suitable extraction solvent for cosmetic products.

3.5 Antibacterial activity

The antibacterial activity against gram-positive bacteria (*S. aureus*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*) of all extracts was determined using an agar well diffusion assay. The gram-positive and gram-negative bacteria were tested with ampicillin and ciprofloxacin as the positive controls. The result showed that all *Z. montanum* extracts gave a quite low efficiency against *S. aureus*, *E. coli*, and *P. aeruginosa*, as shown in Table 3. All the extracts did not inhibit the growth of *S. aureus* at 0.1 mg/mL. Ethanol and 75% ethanol extracts displayed poor inhibition against *E.coli* with a clear zone of 10.5 ± 1.53 and 12.0 ± 0.72 mm and also exhibited insufficient activity against *P. aeruginosa* with a clear zone of 12.0 ± 1.76 and 11.0 ± 0.58 mm.

Table 3. Antibacterial activity of each *Z. montanum* extract

Extract[1]	Zone of Inhibition (in mm diameter)		
	S. aureus	***E. coli***	***P. aeruginosa***
PH	NI	NI	NI
PD	NI	NI	NI
PA	NI	NI	NI
PE	NI	10.5 ± 1.53	12.0 ± 1.76
PM	NI	NI	NI
PE75	NI	12.0 ± 0.72	11.0 ± 0.58
PE50	NI	NI	NI
ampicillin	34 ± 1.82		
ciprofloxacin		35.0 ± 1.14	34.0 ± 1.48

[1] PH: hexane extract, PD: dichloromethane extract, PA: acetone extract, PE: ethanol extract, PM: methanol extract, PE75: 75% ethanol extract, and PE50: 50% ethanol extract, NI: No inhibition zone was observed.

The result indicated that the polar extracts were more active against gram-negative than gram-positive bacteria. The antibacterial properties of *Z. montanum* extracts were comparable to those reported by Aji and colleagues in 2022 [24], who also found 70% ethanol to be the optimal solvent for extraction. However, the difference in the type of bacteria tested was noted. Jena and colleagues reported that the chloroform extract showed significant antimicrobial effects against various pathogens compared to the methanolic extract [25]. However, the age differences, planting locations, and drying methods of the extract led to distinct chemical components and biological activities, differentiating it from this study. The freeze-drying process may potentially remove volatile compounds, such as small terpenoids, which could have antibacterial properties.

The decomposition or evaporation of these terpenoid compounds may result in the remaining substances within the extract having good antioxidant properties but limited antibacterial activity.

3.6 Gas chromatography-Mass spectrometry (GC-MS) analysis

The ethanol extract of *Z. montanum* exhibited high DPPH scavenging activity and strong reducing power. A combined gas chromatograph system and mass spectrophotometer were used to identify its chemical constituents. The analysis of the ethanol extract revealed that it contained a total of 43 volatile compounds, out of which 10 were identified by name at the indicated retention time: 4-methyl-1-(1-methyl ethyl)-bicyclo[3.1.0]hex-2-ene (4.424, 0.42%), terpinene-4-ol (9.285, 0.41%), 4-hydroxy-2-methyl acetophenone (12.905, 0.24%), vanillin (15.125, 0.84%), 3,4-dimethoxybenzaldehyde (17.145, 1.24%), 1,4-dimethoxy-2-methyl-5-(prop-1-en-2-yl)benzene (19.720, 1.64%), 1,4-bis(methoxy)triquinacene (20.733, 19.73%), 2,4,5-trimethoxy-benzaldehyde (22.560, 0.21%), 3-(3,4-dimethoxy phenyl)-2-propenal (23.817, 0.97%), (*E*)-4-(3,4-dimethoxy-phenyl)but-3-en-1-yl acetate (28.172, 11.92%). The major volatile compound is 1,4-bis(methoxy)triquinacene, representing 19.73% of the extract, as illustrated in Figure 1.

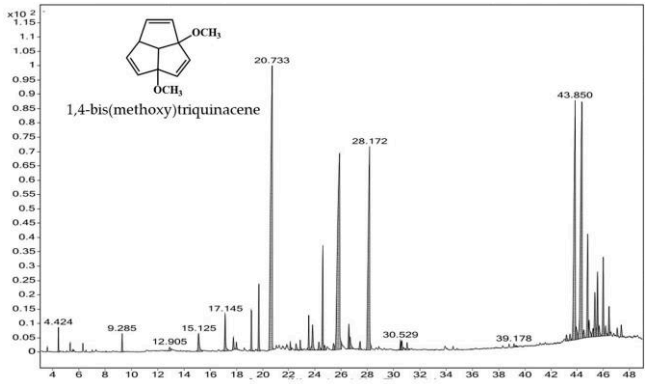

Figure 1. GC-MS analysis of the ethanol *Z. montanum* extract.

The presence of an allylic proton at the fused ring of 1,4-bis(methoxy)triquinacene contributes to its antioxidant properties. This proton can be readily abstracted by the DPPH radical, resulting in the formation of a stable free radical and the generation of DPPH-H, as shown in Figure 2.

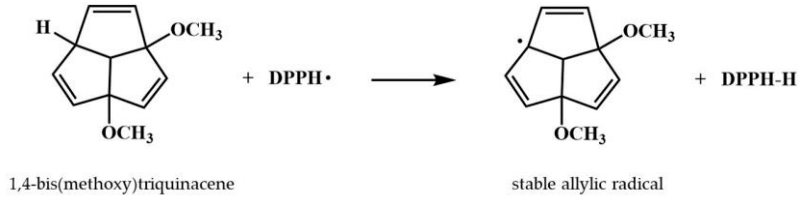

Figure 2. Antioxidant mechanism of 1,4-bis(methoxy)triquinacene with DPPH radical.

4. Conclusions

The suitable solvent for extracting active substances from *Z. montanum* rhizomes for use in cosmetic products was investigated. The rhizomes of *Z. montanum* from Phattalung province, southern Thailand, were extracted using seven types of solvent (hexane, dichloromethane, acetone, ethanol, methanol, 50% ethanol, and 75% ethanol) and freeze-drying. Methanol extract showed the highest phenolic content corresponding to the DPPH radical scavenging activity. At the same time, ethanol extract showed a slightly higher ferric-reducing antioxidant power than methanol extract. All extracts showed poor activity against three tested bacteria strains. Ethanol extract showed the most increased activity against *P. aeruginosa*, whereas 75% ethanol extract showed the highest activity against *E. coli*. The result indicated that ethanol is a suitable solvent for

extracting *Z. montanum* for application in cosmetic products. The major volatile compound present in the ethanol extract is 1,4-bis(methoxy)triquinacene.

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