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Original Article

ANTIAGING CREAM OF *ZINGIBER MONTANUM* RHIZOME EXTRACT THROUGH *IN VITRO* INHIBITION ACTIVITY OF ELASTASE AND TYROSINASE ENZYME

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ABSTRACT

Objective: This study aims to evaluate the elastase and tyrosinase inhibitory activity of bangle (Zingiber montanum) rhizome extract and to develop a cream formulation based on the extract as an anti-aging cosmetic preparation.

Methods: Extraction of Z. montanum rhizome was carried out using 95% ethanol as the solvent, considering its effectiveness in dissolving terpenoid and phenylbutanoid compounds. Metabolite profiling was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) to determine the compound composition of the extract. The anti-aging potential was assessed by in vitro testing for elastase and tyrosinase inhibitory activities. A cream formulation was then developed using extract concentrations of 0.5%, 2.5%, and 5%, and evaluated for physical characteristics including organoleptic properties, emulsion type, homogeneity, pH, viscosity, spreadability, and adhesion.

Results: GC-MS analysis identified 27 compounds in the extract, including terpinen-4-ol and members of the phenylbutanoid group. The bangle rhizome extract demonstrated tyrosinase inhibitory activity with an IC_{50} of 1333.44 mg/L, compared to kojic acid (IC_{50} = 480.72 mg/L). The elastase inhibitory activity showed an IC_{50} of 919.44 mg/L, while the standard gallic acid had an IC_{50} of 325.25 mg/L. The cream formulations prepared with the extract exhibited acceptable physical properties for topical application, including a yellow color with a distinctive aroma, oil-in-water (o/w) emulsion type, and appropriate homogeneity, pH, viscosity, spreadability, and adhesion.

Conclusion: Based on the results, Z. montanum rhizome extract exhibits potential as a natural active ingredient for anti-aging cosmetic products, supported by its elastase and tyrosinase inhibitory activities and its stability and compatibility in cream formulations.

Keywords: *Zingiber montanum*, Antiaging, Elastase, Tyrosinase, GCMS

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INTRODUCTION

The skin is the outermost organ of the body that is the protector of many organ underneath. The increase in the incidence of premature skin aging is a concern for the public and researchers [1, 2]. In addition to natural factors such as age that cause the appearance of skin aging symptoms, external factors such as UV radiation are one of the causes of aging called photoaging. Skin aging can be seen in the form of reduced moisture, elasticity, and the appearance of wrinkles. Hyperpigmentation is also a symptom of photoaging on the skin. UV radiation triggers the formation of reactive oxygen species (ROS), causing oxidative stress in the epidermis of the skin [3, 4]. Antioxidants are needed to suppress ROS, so oxidative stress does not occur quickly, this can prevent premature aging [5]. In addition, ROS can also stimulate elastase enzymes located in the skin layer to break down elastin [6]. Elastin is a protein in the skin that functions to maintain skin elasticity, so that the increased action of the elastase enzyme will be able to accelerate the reduction of skin elasticity, causing symptoms of premature aging such as sagging skin, skin wrinkles, or skin spots [7].

Dark spots on the skin can be a marker of photoaging. The dark color of the skin is related to the process of melanogenesis, which is a process of producing melanin, a black or brown pigment [8]. Although this process is a natural reaction of the skin as a defense against sun exposure, an overreaction can lead to hyperpigmentation. L-tyrosine, which is a non-essential amino acid that constitutes melanin, with the action of the tyrosinase enzyme, will become L-3,4-dihydroxyphenylalanine (L-DOPA). L-DOPA with the enzyme tyrosinase will be converted into L-DOPA-quinone, which then goes through several stages to become melanin [9]. Inhibition of the tyrosinase enzyme is a consideration to reduce the skin pigmentation process, becoming a test in analyzing ingredients that have the potential to whiten or brighten the skin [10, 11].

Indonesia, which is a tropical country with great sun exposure, causes an increase in extrinsic factors that cause premature skin aging due to UV radiation. The discovery of natural plant ingredients that have the potential to be antiaging is an interesting topic to research. The rhizome *of Zingiber montanum* or known by the Indonesian name bangle [12], is empirically used in a mixture of traditional topical preparations, scrubs. The potential of *Z. montanum* has been researched, including as an antiphotoaging, antioxidant, antibacterial [13], anti-inflammatory [14], and immunomodulatory [15, 16]. Phenolic compounds, flavonoids [17], terpenoids, essential oils [18], phenylbutanoids [19] are reported to be contained in *Z. montanum,* showing that *Z. montanum* is a natural ingredient of Indonesian plants that has great potential to be made into topical antiaging preparations. Several studies reported differences in the enzyme inhibitory activity between the essensial oil and 50% ethanol extract, due to differences in compuoud content. Essensial oils contain more terpenoid compounds such as terpinene-4-ol, but 50% ethanol extract of *Z. montanum* rhizome contains phenylbutanoid compounds, causing the difference in activity. Therefore, in this study, 95% ethanol extraction solvent was used, with the hope of providing better enzyme inhibition activity. Inhibition activities of elastase and tyrosinase enzymes were carried out to complete the potential data of the extract and became the basis for the formulation of cream *formulations of Z. montanum* rhizome extract. Cream formulas with different concentrations of extracts are designed to get a good and acceptable picture of the physical characteristics of the cream formulation. Metabolite profiling *of Z. montanum* rhizome extract was carried out to obtain a profile of the compound content in the extract used, thus showing the peculiarities of *Z. montanum rhizome extract.*

MATERIALS AND METHODS

Materials and tools

The main ingredient in this study is bangle rhizome (*Z. montanum*) obtained from the Boyolali area, Central Java, Indonesia. Other

ingredients used are ethanol (Merck), elastase enzyme from porcine pancreas (Sigma – E1250), N-succynil-Ala-Ala-Ala-p-nitroanilide elastase substrate (Sigma – S4760), Trizma Base (Sigma – T1503), Tyrosinase from mushroom (Sigma – T3824), L-Tyrosine (Sigma – T8566), Na-phosphate buffer, kojic acid, and gallic acid. The tools used are GCMS (QP2010S Shimadzu), ELISA reader (BIOTEX Synergi HTX), pH meter, microscope, dispersion test, and adhesion test.

Extract preparation

The rhizomes of *Z. Montanum* are thoroughly washed under running water, chopped and dried in a dryer cabinet with a temperature of no more than 50 °C. Simplisia is then grinded and sifted with a 30/40 mesh. Extraction of 500 g *of Z. montanum* powder using a re-maceration method with 95 % ethanol solvent (2 x 3L) for 4 d. This extraction solvent is used with consideration of the solubility of terpenoid and phenylbutanoid compounds. The extract was then concentrated using a rotary evaporator at a temperature of 40 °C, to prevent degradation of heat-unstable compounds, and the yield is calculated.

TLC test and metabolite profiling using GCMS

As in our previous research, the Thin Layer Chromatography (TLC) test of the extract was obtained by using the silent phase of silica gel F254, the motion phase of n-hexane: ethyl acetate (3:1) with a slight adjustment of the spray reagent used. Observation of spots in visible light, UV 254 nm, UV 366 nm, Liebermann-Burchard spray reagent to determine the content of terpenoid compounds in the extract, and vanillin-H_2SO_4 for essential oil detection. Metabolite profiling of the extract was done using GCMS. The Gas Chromatography-Mass Spectrometry (GCMS) system uses an Agilent HP1 column (30 m x 0.25 mm x 0.25 µm), with EI-70 Ev ionization and Helium as carrier gas. Column temperature 120-300 °C, column rate 0.40 ml/min with split injection 1:33 ratio. The injector and detector temperature are programmed at 310 °C and 305 °C. The metabolite content in the extract was identified using Wiley library data from the GCMS tool, along with the percentage similarity index (SI).

***In vitro* test of elastase enzyme inhibition activity of extract**

The test was carried out using 96-well plates according to the previous method [20] with modifications, divided into Z. *montanum* extract solution in several concentrations, negative control, gallic acid as positive control, and blanks. The solution *of Z. montanum* extract is made by dissolving 20 mg of extract in 1 ml of Dimethyl sulfoxide (DMSO) and adding Triz-HCl buffer (0.1 M pH 8.0) to 10.0 ml, this is done by ensuring that the concentration of DMSO used is no more than 10% Then the extract solution was diluted into test solutions with a concentration of 125-1000 mg/l, this modification of extract concentration was carried out to obtain the IC50 value Galic acid solution was made with a concentration of 1 mmol in Triz-HCl buffer solvent. A total of 10 µl of test solution, 125 µl of N-Suc-(Ala)3-pNA substrate solution (1,015 mmol in Triz-HCl buffer) were put in 96 well plates and pre-incubated for 5 min at 25 °C. Then 15 µl of elastase enzyme from porcine pancreas (0.5 units/ml in cold Triz-HCl buffer) was added. The test solution blank is 10 µl of test solution plus 140 µl of Triz-HCl buffer solution. The negative control consisted of 10 µl of Triz-HCl buffer solution, 125 µl of N-Suc-(Ala)3-pNA substrate solution, and 15 µl of elastase enzyme from porcine pancreas. Then 96 well plates were incubated for 30 min at a temperature of 25 °C, and absorbances were observed at a wavelength of 410 nm. The inhibition activity of elastase enzymes in the form of percent (%) of enzyme inhibition is calculated by the formula {(Absorbances of negative control – Absorbances of test solution)/Absorbance of negative control} x 100. The Inhibition Concentration 50 (IC50), which is the concentration of the extract that can inhibit the elastase enzyme by 50 %, is calculated by the regression equation between the concentration vs the percent of inhibition [21]. Replication was carried out three times.

***In vitro* test of tyrosinase enzyme inhibition activity of extract**

Testing was carried out according to the previous method [22] with adjustments, using 96-well plates. The well plate was divided into Z. *montanum extract* solution in several concentrations, negative control, kojic acid as positive control, and blanks. The solution *of Z. montanum* extract is made by dissolving 20 mg of the extract in 1 ml of DMSO and adding a buffer of Na-phosphate (pH 6.5) up to 10.0 ml. Then the extract solution was diluted into test solutions with a concentration of 15.625-1000 mg/l to obtain the IC50 value. Kojic acid solution was made with a concentration of 2 mmol in a Na-phosphate buffer solvent (pH 6.5). A total of 70 µl of test solution and 30 µl of tyrosinase enzyme from mushroom (333 units/ml in cold Na-phosphate buffer solution) were put in 96-well plates. The negative control consisted of 70 µl of Na-phosphate buffer solution and 30 µl of tyrosinase enzyme from mushroom. Then the 96-well plates were incubated for 5 min, then 110 µl of L-Tyrosine substrate (2 mmol in Na-phosphate buffer) was added. The blanks contain the same solution without the substrate. The 96-well plates were incubated for 30 min at 37 °C and absorbed at a wavelength of 510 nm. The inhibition activity of tyrosinase enzyme in the form of percent (%) of enzyme inhibition is calculated by the formula {(Absorbance of negative control – Absorbance of test solution)/Absorbance of negative control} x 100. The IC50 value, which is the concentration of the extract that can inhibit tyrosinase enzyme by 50 %, is calculated by the regression equation between the concentration vs the percentage of inhibition. Replication was carried out three times.

Z. *montanum* extract cream formulation and physical characteristics test

The cream base is made with ingredients such as stearic acid, cetyl alcohol, Tween 80, Span 80, glycerin, Virgin Coconut Oil (VCO), as well as aquadest. The base is made by mixing the oil part and the water part of the mixture of base ingredients, then stirred until smooth. The cream is made by mixing *Z. montanum* extract with a finished cream base. The concentration of the extract used was 0.5; 2.5; and 5 %. *Z. montanum* essential oil was reported to provide an elastase enzyme inhibition of 27.8% using a 1 mg/ml test solution [23]. *Z. montanum* extract in 96% ethanol solvent (concentration 500 mg/l) was reported to inhibit the enzyme tyrosinase by 15.06% [24]. The study showed that *Z. montanum* can be used as an anti-aging with a concentration of at least 0.05%. The results of preliminary tests of *Z. montanum extract* in our study showed an elastase inhibition of 33.75% using a concentration of 500 ppm, while the inhibition of the enzyme tyrosinase of 15.25% was obtained from an extract with a concentration of 125 ppm. Accordingly, considering an increase in concentration of 10 times from the minimum concentration, which is 0.5% concentration, it will increase the inhibition activity of the enzyme elastase or tyrosinase. Increasing the concentration 5 times and 10 times from the minimum concentration of the extract, which is 2.5% and 5% is then used as a variation of the concentration of the cream formulation.

The cream formulation of *Z. montanum* rhizome extract is made according to table 1. Replication 4 times for each extract concentration used. The physical characteristics test of *Z. montanum* cream formulation was carried out to determine the good preparation and meet the quality requirements of the cream formulation in the form of organoleptics, homogeneity, cream emulsion type, pH, viscosity, spreadability power, and sticking power. The selection of a combination of tween 80 and span 80 as emulsifiers can allow extract that containing of non-polar or polar compounds to be mixed in the cream, and will produce good cream stability.

RESULTS AND DISCUSSION

***Z. montanum* rhizome extract and TLC test**

The results of rhizome extraction *of Z. montanum* were obtained as much as 57.69 g of extract with a yield of 11.54 %. The extract is thick, oily, and yellowish-brown in color. The TLC test of *Z. montanum* rhizome extract (fig. 1. f) showed the content of yellow compounds in visible light (a), compounds with conjugated double bonds so that they were positive with UV light of 254 nm (b) and 366 nm (c), terpenoid compounds that reacted with Liebermann-Burchard spray reagent (d), and essential oil components that appeared as spots with vanillin-H_2SO_4 reagent (e).

Metabolite profiling of *Z. montanum* rhizome extract using GCMS

The GC results showed 27 peaks (fig. 2) with 6 compounds, with a total percentage of 80.39 % of the total 100 % compound content in the extract. The compound content based on the GCMS library data

is shown in table 2. Approximately 20% of the compounds in the extract that are not detected or detected in small amounts can synergize with the major compounds and contribute to the activity of the extract.

Table 1: Cream formula of *Z. montanum* rhizome extract

Ingredients	Function	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)
Z. montanum Extract	Active ingredients	0.5	2.5	5
Cetyl alcohol	Stiffening Agent	4	4	4
Virgin Coconut Oil	Emollient	10	10	10
Tween 80	Emulsifier	2.204	2.204	2.204
Glycerol	Humectant	10	10	10
Span 80	Emulsifier	2	2	2
Methyl paraben	Preservative	0.2	0.2	0.2
Propyl paraben	Preservative	0.1	0.1	0.1
Stearic acid	Emulsifying and solubilizing Agent	3.796	3.796	3.796
Aquadest	Solvent	ad 100	ad 100	ad 100

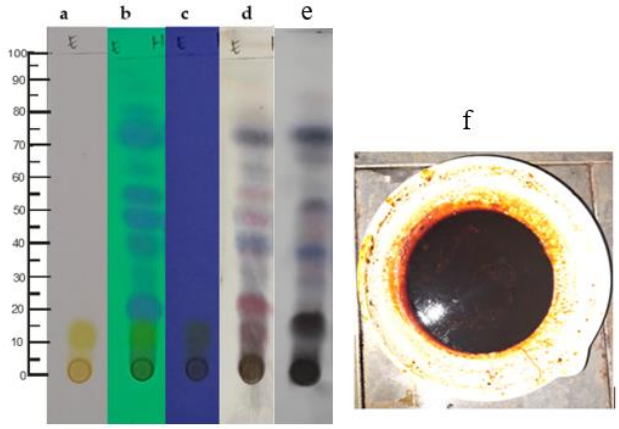

Fig. 1: Chromatogram of TLC test of Z. *montanum* rhizome extract with a stationary phase of silica gel GF 254 and a motion phase of n-hexane: ethyl acetate (3:1). Observations on visible light (a), UV 254 nm (b), UV 366 nm (c), Liebermann-Burchard (d); Vanillin-H_2SO_4 (e); extract *of Z. montanum* rhizome (f)

Table 2: Compound content in *Z. montanum* rhizome extract based on GCMS results

Peak No.	R. Time	Area %	Compound name	SI (%)
1	4.386	3.65	Terpinen-4-ol	98
2	11.381	0.24	Myristicin	69
3	11.921	0.72	2-Methyl-6-(4-metylenecyclohex-2-enyl)-2-Heptene	94
4	13.350	3.58	3-Buten-2-one, 4-(4-hydroxy-3methoxyphenyl)-	72
5	14.720	18.45	2,3,3-Trimetylindolenine	74
6	17.308	0.26	1,2-Methylenedioxy-5,6-dimethoxy-4-allybenzene	73
7	17.592	1.62	Trans-Isomyristicin	74
8	18.499	1.73	3-Ethyl-3-phenyl-2,6-dioxopiperidine	62
9	21.157	0.35	Methyl hexadecanoate	95
10	22.443	13.83	1,4-Diisopropyl-2,5-dimethylbenzene	69
11	22.835	0.43	1-Octadecene	94
12	24.408	1.07	9,12-Hexadecadienoic acid, methyl ester	92
13	29.141	0.29	Trimethyl-tetrahydronaphtalene	64
14	31.704	0.60	Phosponous dichloride, (1,7,7-trimethylbicyclo 2.2.2 hept-2-yl)-	73
15	32.806	0.32	Phosponous dichloride, (1,7,7-trimethylbicyclo 2.2.2 hept-2-yl)-	75
16	33.153	0.77	2H-1-Benzopyran	66
17	33.659	0.28	Podocarpa-1,12-diene-. delta.14,alpha.-acetic acid, 7-hydroxy-8,13-dimethyl-3-oxo-	61
18	34.042	0.36	Dehydroaromadendrene	66
19	38.942	20.59	Benzene, 2-(2-methoxy-1-propenyl)-1,3,5-trimethyl-	68
20	39.692	20.29	Benzene, 2-(2-methoxy-1-propenyl)-1,3,5-trimethyl-	69
21	39.888	2.47	2,5-di-tert-Butylnitrobenzene	65
22	40.281	2.32	2-Imidazolidinone	68
23	40.489	2.06	6-Nitro-5-methoxy-2,3-dimethylindole	66
24	40.933	2.47	5-Nitro-6-methoxy-2,3-dimethylindole	68
25	41.221	0.43	cis-3-(2',4',5'-Trimethoxuphenyl)-4-(E)-2',4',5'-trimethoxystyryl) cyclohex-1-ene	66
26	43.694	0.30	2-Methoxy-3-methyl-1-phenyl-2-buten-1-one	63
27	46.801	0.55	Benzene, 1,3-diethyl-2,4,5,6-tetramethyl-	67

R. time: Retention Time; SI: Similarity Index, Note: compounds in bold indicate large Area %.

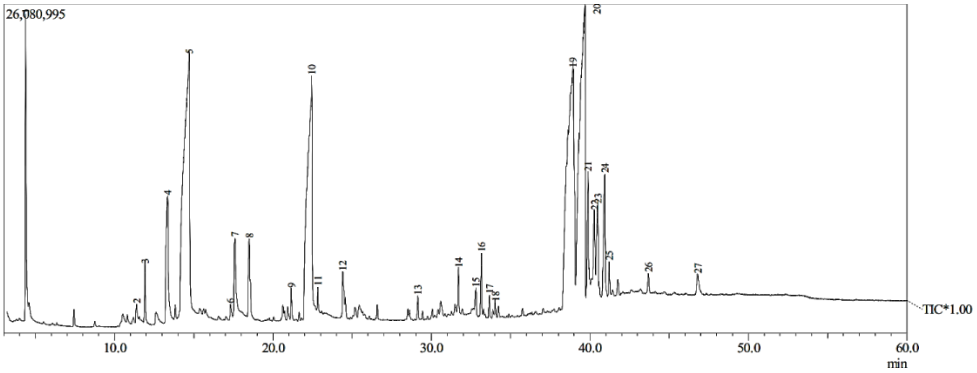

Fig. 2: Chromatogram GCMS rhizome extract Z. *montanum*

Inhibition activity of elastase enzyme of *Z. montanum* rhizome extract

Data on elastase enzyme inhibition activity (fig. 3) with 3 replications obtained an IC50 value of *Z. montanum* (Zm) rhizome extract of 919.44±12.0415 mg/l, gallic acid as a positive control, namely with an IC50 of 325.25±1.8784 mg/l. Phenolic compounds, such as epicatechin, catechin, epigallocatechin, and gallic acid, can significantly inhibit the proteolytic activity of elastase [25]. There is a limitation that gallic acid provides lower activity compared to catechin and epigallocatechin gallate (EGCG). As a reference, the IC50 value of EGCG is 93.99±3.44 µg/ml [26].

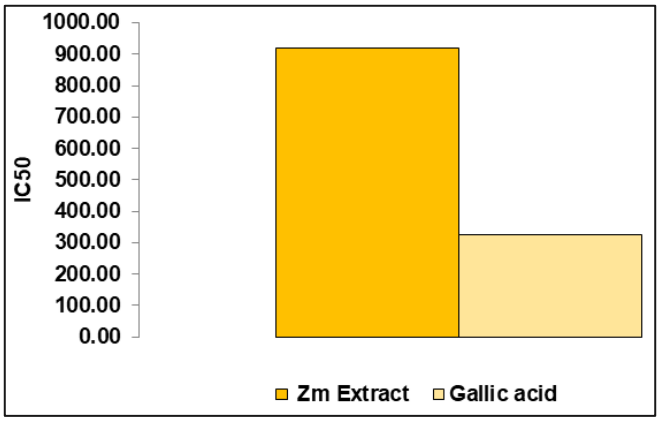

Fig. 3: Inhibition activity of elastase enzyme of *Z. montanum* extract

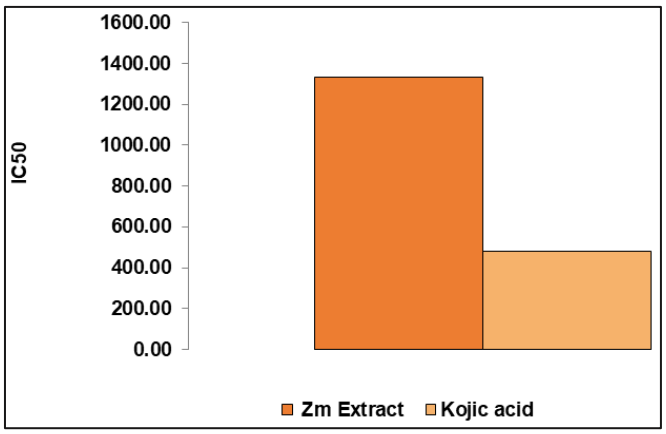

Fig. 4: Tyrosinase enzyme inhibition activity of *Z. montanum* extract

Inhibition activity of tyrosinase enzyme of Z. *montanum* rhizome extract

Data on the inhibition activity of tyrosinase enzyme (fig. 4.) obtained an IC50 value of *Z. montanum rhizome extract* with 3 replications of 1333.44±73.6552 mg/l, inhibition of kojic acid as a positive control, namely with an IC50 of 480.72±2.2681 mg/l. This resulting deviation can be caused by extract variability or test sensitivity using a small number of samples.

***Z. montanum* rhizome extract cream**

The cream extract was made in 4 replicates with extract concentrations of 0.5, 2.5; and 5 %. The results of the physical

characteristics test of the cream formulation are as shown in table 3. The pH of the 0.5 and 5% extract cream did not match the expected pH, which is in accordance with the skin pH, so there is a risk of causing skin irritation. The 5% extract cream exceeds the specified cream spreadability range and may interfere with cream application and comfort of use. All *Z. montanum* rhizome extract creams are in the form of an oil-in-water emulsion and are yellow in color (fig. 5.)

Fig. 5: Cream formulations of *Z. montanum* rhizome extract 0.5; 2.5; and 5 %

Table 3: Physical characteristics of the cream formulation of rhizome *extract Z. montanum*

Physical characteristics	Result of cream			Requirements
	0.5 %	2.5 %	5 %	
Organoleptic	light yellow cream with aromatic smell	yellow (+) cream with aromatic smell	yellow (++) cream with aromatic smell	
Cream emulsion type	o/w	o/w	o/w	
Homogeneity	homogeneous	homogeneous	homogeneous	
pH	4.368	4.585	4.242	Requirement: 4.5-6.5 [27]
Viscosity (cP)	4415	4610	4561	Requirement: 4000-40000 cP [28]
Spreadability (cm)	6.65	6.97	7.72	Requirement: 5-7 cm [29]
Sticking power (seconds)	1.95	1,29	1.46	

DISCUSSION

In accordance with the results of the TLC test, *Z. montanum* rhizome extract contains several terpenoid compounds, with metabolite profiling using GCMS as shown in table 1. Terpenoid content in the form of terpinen-4-ol compounds (RT 4,386 min) is reported to be found in essential oils [30] or ethanolic extracts of *Z. montanum* rhizomes detected with GCMS [31]. Phenylpropanoid components are also reported to be contained in essential oils of the Zingiberaceae family as well as the content of several phenylbutanoids in *Z. montanum* [32]. The compounds 3-Butene-2-one, 4-(4-hydroxy-3methoxyphenyl)-(RT 13,350 min), 2,3,3-Trimethylindolenine (RT 14,720 min), 1,4-Diisopropyl-2,5-dimethylbenzene (RT 22,443 min), Benzene, 2-(2-methoxy-1-propenyl)-1,3,5-trimethyl (RT 38,942 min and 39,692 min) are other major compounds in the extract in the form of phenylpropanoids and phenylbutanoids. The extraction solvent used in this study was 95% ethanol, which, with small water content can dissolve semipolar compounds such as phenyl butanoids in addition to non-polar terpenoid compounds from *Z. montanum* rhizome.

The curcuminoid compound reported to be contained in the rhizome extract *of Z. montanum* [33] was not detected in the GCMS test, but it can be seen as a yellow spot in the TLC test results (fig. 1-a). Non-volatile curcuminoids could not be detected with GCMS in this study, therefore in futher studies, identification can be carried out with HPLC or LCMS, which are more suitable for non-volatile compounds. Compounds such as terpinen-4-ol, phenylbutanoids, and curcuminoids have antioxidant and anti-inflammatory functions [34]. This antioxidant function directs *Z. montanum* to potentially inhibit elastase and tyrosinase enzymes.

Another study reported that *Z. montanum* essential oil with a concentration of 1 mg/ml was reported to provide elastase enzyme inhibition activity of 27.80±2.11 %, in contrast to the tyrosinase enzyme inhibition test, which showed no inhibition activity [24]. *Z. montanum* has the potential to suppress the formation of matrix Metaloproteinases (MMPs) of both essential oils [24] and extracts [31], which together with the ability to inhibit elastase enzymes, are closely related to the potential *of Z. montanum* as a protective agent against photoaging. Elastase enzyme, which is one of the protease enzymes that functions to break down elastin, a protein that maintains skin elasticity, causes the skin to look aged due to wrinkles. The inhibition activity of elastase enzyme of *Z. montanum* rhizome extract in this study showed a smaller IC50 value (919.44±12.0415 mg/l) compared to the reported *Z. montanum* rhizome extract [35] with an IC50 of 3386.23 µg/ml, using 50 % ethanol solvent. The greater inhibitory ability of elastase enzymes in this study was related to active compounds that could be extracted better with 95 % ethanol solvents compared to 50 % ethanol. The antioxidant compounds in the rhizome *of Z. montanum* are extracted less and less in ethanol solvents with the addition of water, thereby decreasing its antioxidant activity [13], which plays a role in the inhibition of elastase enzymes.

The results of the enzyme elastase inhibition test showed that the IC50 of *Z. montanum* extract was 9 to 10 times greater than EGCG, which is commonly used as a standard in antiaging formulations. EGCG was reported to have an IC50 value of 93.99±3.44 [26] or 110.00±2.41 ppm [36]. Green tea extract of 1% equivalent to EGCG 0.006% (w/w) in an oil-in-water emulsion has been reported to reduce wrinkles and can brighten skin in clinical trials on facial skin of respondents [37]. Although further research is needed, the 0.5% *Z. montanum* extract in preparations may be a promising antiaging cosmetic ingredient.

Although other study has reported that Z. *montanum* rhizome essential oil does not have tyrosinase enzyme inhibition activity, in this study Z. *montanum* rhizome extract showed inhibition activity (fig. 4). Interestingly, this is similar to the results of the study [35] which showed inhibition activity with an IC50 of 1373.68 µg/ml from an extract with 50 % ethanol solvent. Furthermore, in the study [14] it was reported that the content of compounds (*1E,4E,6E*)-1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one separated from ethyl acetate extract *Z. montanum* was able to inhibit tyrosinase enzyme by 42.56±1.02 % with a concentration of less than 100 µg/ml. The presence of conjugated double bonds and hydroxy groups, as well as those also found in curcuminoids, is assumed to exert an influence on tyrosinase inhibition [38].

The ability of *Z. montanum rhizome extract* in inhibiting elastase and tyrosinase, and supported by the results of our previous research [39], the development of a topical preparation formula in the form of an antiaging cream from *Z. montanum* rhizome extract is interesting to do. All cream formulations made in this study are in the form of an oil emulsion in water, yellow in color, and have a characteristic aroma of *Z. montanum* rhizome, homogeneous, and meet the viscosity requirements of cream preparations. However, only the 2.5 % Z. *montanum* rhizome extract cream formulation meets the cream formulation requirements for pH. The pH of 0.5 and 5% extract creams did not match the expected pH. This pH deviation is possible due to the reaction of the extract content with the cream ingredients, so the use of buffer agents is a consideration for the further formulation. The pH of the extract as well as the ingredients used as the cream base, affect the pH of the formulation. The composition of the extract and ingredients in the base of the 2.5 % extract cream formulation produces a pH that corresponds to the compatibility and stability of the skin [40], this makes the value more than the 2.5 % extract cream formulation. The viscosity of the cream is related to the spread and adhesion of the cream, the thicker the cream, the smaller the spread, but the greater the sticking power [28]. Spreadability and adhesion are closely related to the quality of the formulation, the cream will be able to spread easily and will not be sticky when applied to the skin.

CONCLUSION

Compounds contained in the rhizome *of Z. montanum,* such as terpinen-4-ol, phenylbutanoids, or phenylpropanoids are compounds that have antioxidant, anti-inflammatory, and antiphotoaging potential. This study complements previous research on the potential of *Z. montanum* rhizome extract for cosmetics in the form of elastase and tyrosinase enzyme inhibition activities. It is undeniable thar there are limitation in this study related to the absence of in vivo tests, safety, and stability test of cream undeniable than formulation. Moderate enzyme inhibition ability is the basis for the development of antiaging cream formulations *of Z. montanum rhizome extract* with good physical characteristics of the formulation. Furthermore, the use of extract concentration in cream requires evaluation of *in vivo* activity and further stability testing of cream formulations.

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AUTHORS CONTRIBUTIONS

Endang Dwi Wulansari: Conceptualization, Methodology, Writing – Original Draft, Supervision. Wulandari: Resources, Writing – Review and Editing, Funding acquisition. Fransisca Miranda Puspita Sari: Investigation, Visualization. Endang Diyah Ikasari: Validation, Formal Analysis, Project administration.

CONFLICTS OF INTERESTS

The authors report no conflicts of interest in this work.

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