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Potential of *Curcuma zedoaria* Hydrosol as an Antioxidant and Active Ingredient for Emulgel

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ABSTRACT

Hydro-distillation of *Curcuma zedoaria* rhizomes produces essential oils with high antioxidant activity. The distillation process results in a large amount of residue (hydrosol) which are underutilized. Hydrosol contains 0.2% of essential oil which can be harnessed into high value products. This study aimed to assess the antioxidant potential of *Curcuma zedoaria* hydrosol, as well as its emulgel formulations. *Curcuma zedoaria* hydrosols were obtained from hydro-distillation at 100°C for 3 hours. The chemical constituents of the hydrosols were determined by gas chromatography-mass spectrometry (GC-MS). The hydrosol was formulated into emulgels containing different hydrosol concentrations; F1 (30%), F2 (40%), and F3 (50%). The physicochemical properties of the emulgels were evaluated according to standard procedures. Antioxidant activity of both the hydrosol and emulgels was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The GC-MS analysis showed that the major components of *Curcuma zedoaria* hydrosols were 1, 4-cineole, bicyclo [2.2.1] heptane, 2-propanone, and 2-propynoic acid. The hydrosol and emulgels demonstrated a concentration-dependent antioxidant activity with IC_{50} values of 92.31 ± 4.96 g/L, 218.62 ± 22.37 g/L (F1), 159.86 ± 10.46 g/L (F2), and 133.13 ± 25.85 g/L (F3). All three emulgel formulations were yellowish-white semi-solid with a distinctive smell of *Curcuma zedoaria* and pH between 4.59 – 4.76. All other physical parameters were within acceptable limits. These findings suggest that *Curcuma zedoaria* hydrosol has great potential to be developed as active ingredient in topical skincare products.

Keywords: *Curcuma zedoaria*, Antioxidant, Hydrosol, Emulgel.

Introduction

Since the covid-19 pandemic, public demand for traditional medicine has continued to increase. In addition, the government has continued to encourage the development of medicinal and cosmetic products from natural ingredients to reduce over-reliance on imported synthetic drugs. The genus *Curcuma* of the *Zingiberaceae* family is reported to have beneficial effects on the skin. One important species in this genus is *Curcuma zedoaria*. This plant has been shown to possess numerous biological activities, including anti-aging, antioxidant, anti-inflammatory, and UVB-protecting effects.[1,2] One of the main components in *Curcuma* rhizome is the volatile oil (essential oil) which is responsible for most of the biological activities of the plant.[3] The production of essential oils using distillation often produce a lot of hydrosols (distillation residue) which is underutilized. Hydrosols from plants such as Lemongrass has been reported to have significant therapeutic benefits, particularly antioxidant potential.[4] Although, the antioxidant activity of *Curcuma zedoaria* hydrosol has not been reported, its extract and essential oil have been studied for their potential antioxidant and anti-inflammatory activities.[5-7]

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The antioxidant activity of the essential oil has been shown to surpass those of well-known antioxidants such as butylated hydroxyanisol (BHA), alpha-tocopherol, and ascorbic acid.[8]Some of the beneficial compounds that are thought to be present in the hydrosol of *Curcuma zedoaria* include sesquiterpenes such as curcumin, ethyl p-methoxycinnamate, *β*-turmeron, *β*-eudesmol, zingiberene, dihydrocurcumin, furanodiene, α-phellandrene, 1,8-cineole, *β*-elemene, and germacrone.[3,9] The presence of antioxidant compounds in *Curcuma zedoaria* hydrosol can help stabilize free radicals and can accelerate cell regeneration when used topically.[1] Therefore, it is necessary to explore the benefits of hydrosol waste to increase its economic value as a drug or cosmetic product.

Emulgel is a topical drug delivery system that combines the properties of both an emulsion and a gel, resulting in a formulation that produces a pleasant and comforting experience when applied to the skin. This preparation facilitates drug penetration into the skin and provide faster drug action, better dispersion, and a longer shelf life.[10] The addition of *Curcuma zedoaria* hydrosol as an active ingredient in emulgel has the potential to improve its bioactivity as antioxidant, and this formulation is expected to increase the use value of *Curcuma zedoaria* essential oil processing by-product.

So far, the formulations derived from *Curcuma zedoaria* essential oil include emulgel, serum, and SNEEDS (Self-Nanoemulsifying Drug Delivery System). Emulgels are bio-friendly, thixotropic, greaseless, easily spreadable, easily removable, have extended shelf life and have good acceptable appearance.[11,12] *Curcuma zedoaria* extract on the other hand have been used in many other formulations like creams, gels, serums, microemulsions, and body scrub.[13] The aim of the present study was to investigate the physicochemical properties and antioxidant activity of *Curcuma zedoaria* hydrosol and emulgel formulations.

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Materials and Methods

Chemicals and Equipment

Carbopol 940 (Corel Pharma Chem, INDIA), DPPH *(1,1-diphenyl-2-picrylyhydrazol)* (Smart Lab, Indonesia), methylparaben and propylparaben (Ueno, Japan), liquid paraffin and triethanolamine (TEA) (Petronas, Malaysia), tween 80 and span 80 (KAO corporation, Japan), ethanol (Merck, Germany). The equipment used include digital analytical balance (Fujitsu FS-AR, Japan), magnetic stirrer, vortexer (Gemmy VM-300, Taiwan), pH meter (OHAUS type AB33PH-F, USA), UV-Vis Spectrophotometer (Shimadzu UV-1780, Japan), GC-MS (GCMS-QP2010S, Japan), Centrifuge (Model No. 80-2, China). All glassware were products of pyrex.

Plant collection and identification

The rhizomes of the *Curcuma zedoaria* were collected in February 2024 from Karangpandan, Karanganyar, Central Java, Indonesia. The plant material was identified at Center for Research and Development of Medicinal Plants and Traditional Medicines with voucher number KM.04.02/2/426/2023.

Distillation of Curcuma zedoaria rhizomes

Curcuma zedoaria rhizomes were cleaned, peeled, and 200 g of the rhizomes were placed in a distillation flask (A), followed by the addition of 750 mL of distilled water. The sample was distilled at 100℃ for 3 hours and the distillate (hydrosol) was collected in a separate flask (B).[4]

GC-MS analysis of Curcuma zedoaria hydrosol

The volatile compounds in the *Curcuma zedoaria* hydrosol were determined by gas chromatograph coupled to a mass spectrometer with manual injection (Shimadzu GCMS-QP2010S, Japan). The column used was a RESTEK Rx-1MS 30 m long, 0.25 mm internal diameter, and a film thickness of 0.25 µm. Injections were carried out in split mode (ratio 1:10). Helium was used as the carrier gas at a flow rate of 1 mL/min. Injection temperature were raised to 200℃. The injection volume was 1 µL. Analyses were carried out in electron impact ionization (EI) mode with an ionization energy of 70 eV using scan mode acquisition in m/z range from 35 to 600 amu. Compounds were identified by comparing the retention times of the samples and standard solutions, then comparing the mass spectra with those contained in the NIST® commercial library.[14]

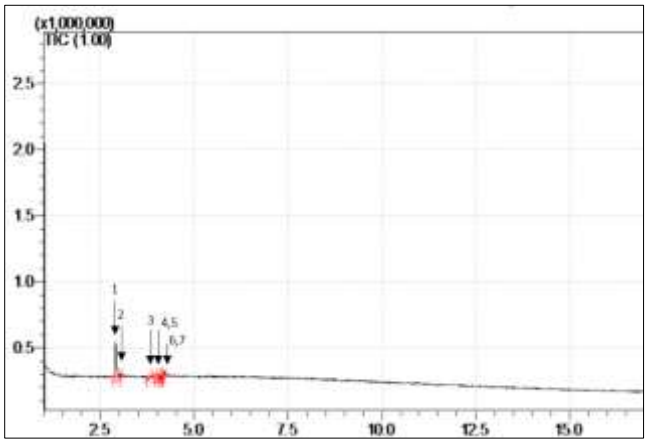

Figure 1: GC chromatogram of *Curcuma zedoaria* Hydrosol in Flask A

Preparation of Curcuma zedoaria hydrosol emulgel

Three emulgel formulations (F1, F2, and F3) containing different proportions of *Curcuma zedoaria* hydrosol were prepared according to the formula presented in Table 1. First, the gelling agent (carbopol 940) was mixed with 10 times its weight of water, and allowed to swell. After swelling, the pH of the gel was adjusted to 7 by the addition of triethanolamine (TEA). Thereafter, the oil phase was prepared by mixing span 80 and liquid paraffin. The water phase which consists of the preservatives and hydrosol was prepared by dissolving a mixture of methylparaben, propylparaben and *Curcuma zedoaria* hydrosol in propylene glycol and tween 80. Then, the oil phase and water phase were mixed to form an homogeneous phase, which was then transferred into the previously prepared gelling agent (carbopol 940), and the final mixture was thoroughly mixed until a homogeneous emulgel was formed.

Determination of the physicochemical properties of the emulgel

The physicochemical properties of the emulgel were determined in order to assess its quality in comparison with other commercial emulgel products. The physicochemical tests carried out include organoleptic test, dispersion/spreadability test, adhesion test, viscosity test, and pH and sensitivity test. The organoleptic test was carried out through direct observation of the colour, odour, and form of the preparation. Spreadability test was carried out using two petri dish lids that were loaded with weights of 50 - 250 g for 1 minute. Viscosity test was performed using the Brookfield type viscometer with spindle. pH was determined using a pH meter (AB33PH-F, OHAUS, USA).[15]

Table 1: Emulgel Formula

Ingredient	Function	Composition (%)		
		F1	F2	F3
Curcuma zedoaria hydrosol	Active Ingredient	30.0	40.0	50.0
Carbopol 940	Gelling agent	0.5	1.0	1.5
Triethanolamine (TEA) pH 7	Buffer	Qs	Qs	Qs
Liquid paraffin	Base	5.0	5.0	5.0
Span 80	Emulsifier	5.0	5.0	5.0
Tween 80	Emulsifier	5.0	5.0	5.0
Propylene glycol	Enhancer	10.0	10.0	10.0
Methylparaben	Water phase preservative	0.6	0.6	0.6
Propylparaben	Oil phase preservative	0.3	0.3	0.3
Distilled water	Solvent	Qs add 100*	Qs add 100*	Qs add 100*

**Qs add 100 means add distilled water until 100% composition*

Determination of antioxidant activity of hydrosol and emulgel

The antioxidant activity of hydrosol and its emulgel formulations (F1, F2, and F3) was determined via the DPPH free radical scavenging assay.[16] Stock solution of DPPH (100 ppm) was diluted to 50 ppm with ethanol. The absorbance of the solution was measured through 450-650 nm to determine the wavelength of maximum absorption (λmax). The operating time for the radical scavenging assay was determined by mixing 4 mL of 50 ppm DPPH solution and 1 mL of hydrosol and determining the absorbance at the predetermined wavelength every 5 minutes for 1 hour.[17]

Concentration series (15, 20, 25, 30, 35, and 40 ppm) of the hydrosol and emulgel were prepared in ethanol. Each of the test solutions (1 mL) was added to 4 mL of DPPH (20 ppm) solution, and the mixture was incubated in a dark room at room temperature for 105 minutes operating time. The percentage of radical inhibition was calculated using the formula below (Equation 1).[18]

$$Inhibition\ (\%) = \frac{Abs\ of\ control - Abs\ sample}{Abs\ of\ control} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

The IC_{50} was calculated from the linear regression equation obtained from the plot of percentage inhibition versus concentration. The experiments were performed in triplicate.

Statistical analysis

Antioxidant data were presented as Mean ± SD. Differences between mean values were analyzed using one-way analysis of variance (ANOVA) followed by Tukey HSD test. $p < 0.05$ was regarded as significant.

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Results and Discussion

Distillation yield of Curcuma zedoaria rhizomes

During the distillation process, two products were produced, namely essential oils and the by-products in the form of hydrosols. From 200 g of fresh *Curcuma zedoaria* rhizomes distilled with 750 mL of distilled water at a temperature of 100℃ for 3 hours, 89 mL of hydrosol in distillation flask (A) and 636 mL of hydrosol in distillation flask (B) were obtained. The hydrosol of *Curcuma zedoaria* in flask B was appeared as a fresh yellow liquid with a distinctive smell of *Curcuma zedoaria*. Meanwhile, the hydrosol in flask A was clear, colourless, and has a typical aroma of *Curcuma zedoaria*. Studies have shown that the chemical constituents of essential oils are essentially same as those of hydrosols and has similar pharmacological activity.[19–21]

Chemical constituents of Curcuma zedoaria hydrosol

The chemical constituents of hydrosols were identified by GC-MS analysis (Figure 1 and 2). The chemical constituents of the hydrosols are presented in Tables 2 and 3. The major components of the hydrosol in flask A were 1, 4-cineole and bicyclo [2.2.1] heptane, while, the hydrosol in flask B contained mainly 2-propanone and 2-propynoic acid.

The content of flask A hydrosol was dominated by terpenoid compounds (Table 2). This is similar to the findings from a previous study which showed that the main constituents of *Curcuma* species essential oil were terpenoids such as *β*-pinene (21.64%), and *β*-myrcene (43.78%), while a small fraction were other compounds such as cyclohexane (4.84%); geranylgeraniol (0.50%); caropylene oxide (0.58%).[3] In this study, hydrosol in flask B was used in the determination of antioxidant activity and the formulation of emulgel.

Table 2: Chemical constituents of *Curcuma zedoaria* hydrosol in Flask A

Peak No.	Retention Time (minutes)	Peak Area (%)	Compound Name
1	2.897	59.2	1,4-Cineole
2	3.021	5.21	2-Amino-N-methylpropanamide
3	3.81	5.76	3,5,8(2*H*)-Isoquinolinetrione
4	3.99	7.14	4-Octen-3-one
5	4.069	4.47	1*H*-Indole
6	4.135	4.94	Alanine
7	4.187	13.28	Bicyclo[2.2.1]heptane

Table 3: Chemical constituents of *Curcuma zedoaria* hydrosol in Flask B

Peak No.	Retention Time (minutes)	Peak Area (%)	Compound Name
1	1.176	16.41	2-Propynoic acid
2	1.221	58.43	2-Propanone
3	1.35	7.12	2-Hexanamine
4	1.388	5.85	2-Acetylaminophenyl ester
5	1.454	3.59	2-Pentanamine
6	1.555	2.91	5-Hydroxydihydro-2,4(1*H*,3*H*)-Pyrimidinedione
7	1.591	5.7	Dextroamphetamine

Antioxidant activity of Curcuma zedoaria hydrosol

The determination of antioxidant activity began with the determination of the wavelength of maximum absorption (λmax) of DPPH. As shown in Figure 3, the λmax of DPPH (50 ppm) was found to be 514 nm. This wavelength was subsequently used for the determination of the antioxidant activity of *Curcuma zedoaria* hydrosol.

Secondly, the optimal operating time of DPPH-Hydrosol interaction was determined based on the time the absorbance value began to stabilize or the difference in absorbance value at each time interval began to decrease.[22] The operating time (OT) obtained for the test solution was 105 minutes, and this was characterized by a stable absorbance value. The results of the antioxidant activity test indicated that *Curcuma zedoaria* hydrosol demonstrated a concentration-dependent increase in antioxidant activity (Table 4). The antioxidant activity was expressed in terms of the IC_{50} value. The IC_{50} is a parameter used to express the ability of a test sample to inhibit DPPH radical activity by 50%. Table 4 shows that *Curcuma zedoaria* hydrosol has potential antioxidant activity as evidenced by the average IC_{50} value of 92.31 ± 4.96 g/L which is equivalent to 9.2% of the hydrosol when used for topical preparations. It has been shown that IC_{50} of hydrosol is generally higher than that of the extract and essential oil.[23] Despite having high IC_{50} value, *Curcuma zedoaria* hydrosol has great potential to be developed as an active ingredient in topical preparations. In line with the findings from other studies on the antioxidant activity of hydrosols, it has been shown that distillation residues or hydrosols from various parts of plant have varying antioxidant activity.[4,24]

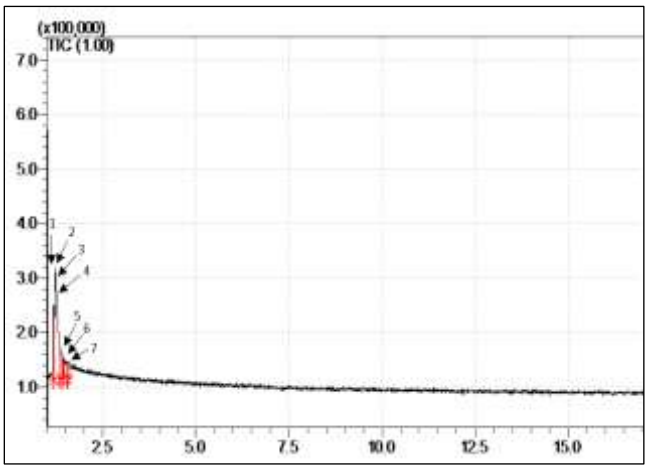

Figure 2: GC chromatogram of *Curcuma zedoaria* Hydrosol in Flask B

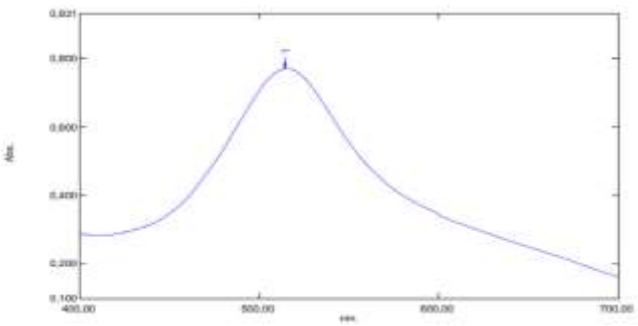

Figure 3: Visible spectrum of DPPH (50 ppm) showing the λmax

Physicochemical properties of Curcuma zedoaria hydrosol emulgel

The emulgel preparation was made using three formulas with the emulgel compositions in each of the formulas being 30% (F1), 40% (F2), and 50% (F3). The physicochemical properties evaluated include organoleptic properties, homogeneity, pH, viscosity, adhesiveness, and dispersivity. The results of organoleptic observations show that the emulgel produced was yellowish-white and has a distinctive odour of

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Curcuma zedoaria. The emulgels were semi-solid with F3 appearing most viscous, followed by F2, and F1 appearing least viscous. All three formulation were homogeneous in terms of texture and colour. The pH of the three formulations were 4.59, 4.68, and 4.76 for F1, F2, and F3, respectively (Table 5), which met the standard pH requirement of 4 – 6 for skincare products.[25]

The viscosity data of all three formulations are presented in Table 5. The amount of hydrosol added to the emulgel preparation did not have a significant effect on its viscosity. The acceptable range of viscosity value in emulgel preparations is 1000 – 10,000 cps.[25]

The adhesion test aims to find out how long the emulgel preparation can stick to the skin. Based on the data in Table 5, it was found out that the more the hydrosol added to the emulgel, the less the adhesion time.

The dispersibility test was carried out with the aim of determining the ability of the formulation to disperse when applied to the skin and to find out whether the active substance can be dispersed evenly or not on the skin, which is a measure of its ability to provide a uniform and maximum effect. Good dispersibility should be within the range of 5 - 7 cm. The results as shown in Table 5, indicate that the addition of hydrosol to the emulgel preparation has no significant effect on the dispersibility of the preparation. All three formulations met the dispersibility requirements of emulgel preparations.

Table 4: Antioxidant activity of *Curcuma zedoaria* hydrosol

Concentration (ppm)	Inhibition (%) of DPPH radical			Average Inhibition (%)
	Replicate 1	Replicate 2	Replicate 3	
65	43.6	38.92	44.17	42.23 ± 2.88
85	50.14	48.48	45.15	47.92 ± 2.54
105	53.56	53.17	53.40	53.37 ± 0.19
125	60.39	60.48	60.59	60.48 ± 0.10
145	63.60	59.21	63.99	62.26 ± 2.65
165	65.46	65.85	62.55	64.62 ± 1.80
185	71.41	68.00	66.86	68.75 ± 2.37
IC_{50}	87.49 ppm	97.40 ppm	92.05 ppm	
Average IC_{50}	92.31 ± 4.96 ppm			

Table 5: Physicochemical properties of *Curcuma zedoaria* Hydrosol Emulgel Formulations

Parameter	Value		
	F1	F2	F3
pH	4.59	4.68	4.76
Viscosity (mPas)	9816.5	9815.3	9815.3
Adhesion (s)	5.02	4.13	4.11
Dispersibility (cm)	6.49	6.45	6.26

Antioxidant activity of the emulgel

The ability of the hydrosol emulgel to scavenge DPPH free radicals was observed qualitatively by the change in colour of DPPH solution from purple to brown to yellow. This occurs due to the ability of DPPH radical to accept hydrogen atom from antioxidant compound, and be reduced to DPPH-H.[26] The result of the antioxdant test of *Curcuma zedoaria* hydrosol emulgel is presented in Table 6.

Table 6: Antioxidant activity of *Curcuma zedoaria* hydrosol emulgel

Formula	Replicate	IC_{50} value (g/L)	Average IC_{50} (g/L)
F1	1	195.82	218.62 ± 22.37
	2	219.52	
	3	240.53	
F2	1	171.71	159.86 ± 10.46
	2	151.90	
	3	155.98	
F3	1	117.11	133.13 ± 25.85
	2	119.33	
	3	162.96	

It was observed that the concentration of *Curcuma zedoaria* hydrosol in the emulgel formulation affected its antioxidant activity. The higher the concentration of hydrosol as the active ingredient in the emulgel, the more compounds it contains that can increase the antioxidant activity of the emulgel and vice versa. In this study, the highest antioxidant activity was found in formulation F3 of *Curcuma zedoaria* hydrosol emulgel containing 50% *Curcuma zedoaria* hydrosol with an IC_{50} value of 133.13 ± 25.85 g/L.

Conclusion

Curcuma zedoaria hydrosol was successfully formulated into emulgel with varying concentrations of the hydrosol; 30% (F1), 40% (F2), and 50% (F3). Although, the antioxidant activity of the emulgel formulations was lower than that of the hydrosol, their physicochemical properties including organoleptic properties, homogeneneity, pH, viscosity, dispersibility, and adhesiveness met the requirements for emulgel formulations. These observations indicate that *Curcuma zedoaria* hydrosol has great potential to be developed as active ingredient in topical skincare products.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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