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Effect of Solvent Polarity on Terpenoid Content and Antioxidant Capacity of *Curcuma xanthorrhiza* Rhizome Extracts

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Abstract: *Curcuma xanthorrhiza* is a traditional medicinal plant known for its antioxidant and terpenoid content. This study aimed to identify the most effective solvent for extracting antioxidant compounds and terpenoids from *the rhizomes of C. xanthorrhiza*. Extraction was carried out using sonication followed by maceration, employing four solvent systems: hexane, acetone, a mixture of hexane and acetone, and a mixture of hexane and ethyl acetate. Terpenoid content was quantified using a vanillin-perchloric acid method, and antioxidant capacity was assessed using DPPH, ABTS, FRAP, and CUPRAC assays. Hexane yielded the highest total terpenoid content (243.74 ± 8.13 mg UA/g DW), while acetone showed superior antioxidant activity, particularly in the FRAP assay (131.25 ± 4.33 µmol TE/g DW). These findings suggest that solvent polarity has a significant impact on extraction efficiency and that terpenoids may not be the primary contributors to antioxidant activity in *C. xanthorrhiza*. Phenolic and flavonoid compounds, known to be more soluble in polar solvents such as acetone, might be the primary antioxidants. Therefore, the choice of solvent should consider both the target compounds and practical applications, including pharmaceutical and nutraceutical uses. Further investigation is required to identify the primary antioxidant constituents responsible for the observed activity.

Keywords: *Curcuma xanthorrhiza*; solvent polarity; terpenoid content; antioxidant capacity; DPPH; ABTS; FRAP; CUPRAC.

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1. Introduction

Antioxidants are molecules that prevent or slow oxidative damage caused by free radicals. These reactive oxygen species are naturally generated as byproducts of cellular metabolism and, in excess, can disrupt physiological functions and damage biomolecules [1,2]. Antioxidants exert their protective effects primarily by donating electrons or hydrogen atoms to neutralize free radicals, thereby inhibiting oxidative stress and subsequent cellular damage [3–5]. While the body produces endogenous antioxidants, they are often insufficient to counterbalance oxidative stress, necessitating the use of external sources, such as foods, supplements, or herbal medicines [6].

Curcuma xanthorrhiza Roxb., commonly known as Javanese turmeric or "temulawak," is a tropical medicinal plant native to Indonesia and classified under the Zingiberaceae family. It has long been used in traditional medicine due to its wide range of pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, anti-aging, and antitumor activities [7,8]. The rhizome of *C. xanthorrhiza* contains various bioactive constituents such as curcumin, terpenoids, flavonoids, phenolics, and essential oils [9,10]. Among these, terpenoids are particularly notable for their diverse biological activities, including antioxidant, anticancer, and antimicrobial effects [11–13]. Identified terpenoids in *C. xanthorrhiza* include α-curcumene, xanthorrhizol, germacrone, and α-turmerone [7]. However, recent studies suggest that phenolic compounds, including curcumin and other flavonoids, may primarily drive antioxidant activity in this plant.

Due to its medicinal significance and increasing demand, *C. xanthorrhiza* is recognized as one of Indonesia's priority medicinal plants and a key biopharmaceutical commodity [14,15]. Efficient extraction of its bioactive compounds is crucial for meeting industrial and pharmaceutical needs. Solvent selection is a crucial factor influencing the yield and composition of extracted compounds, as it affects solubility based on the polarity of the compounds [16]. Nonpolar solvents such as hexane are generally more effective in extracting lipophilic compounds like terpenoids, while polar and semi-polar solvents such as acetone or ethyl acetate target a broader range of antioxidant constituents [17–19].

This study aimed to evaluate the effect of different solvents—hexane, acetone, hexane-acetone, and hexane-ethyl acetate—on the extraction efficiency of terpenoids and antioxidant activity from *C. xanthorrhiza* rhizomes. The results are expected to identify the most effective solvent system and clarify the role of terpenoids in contributing to the overall antioxidant activity of the plant.

2. Materials and Methods

2.1. Plant material and preparation of simplisia.

Fresh rhizomes of *Curcuma xanthorrhiza* were obtained from the Cultivation Conservation Unit of the IPB Biopharmaca Garden, Tropical Biopharmaca Research Center (TropBRC), IPB University, Bogor, Indonesia. The rhizomes were washed, sliced, and dried in a hot-air oven at 45°C for 48 hours. The dried rhizomes were ground and passed through an 80-mesh sieve to obtain a uniform powder (simplisia), which was used for further extraction.

2.2. Extraction procedure.

Four types of solvent systems were used: hexane (H), acetone (A), hexane-acetone (HA, 1:1 v/v), and hexane-ethyl acetate (HEA, 1:1 v/v). A total of 4 g of *C. xanthorrhiza* rhizome powder was extracted with 40 mL of the designated solvent. Extraction was performed by ultrasonic treatment using a Decon F5 Major sonicator (Decon Laboratories, USA) for 30 minutes, followed by maceration in a WiseBath water bath shaker (Daihan Scientific, Korea) for 180 minutes. Figure 1 illustrates the schematic workflow of the extraction procedure. After filtration through Whatman No. 1 filter paper, the volume of each extract was adjusted to 20 mL if necessary to achieve a final concentration of 0.2 g/mL.

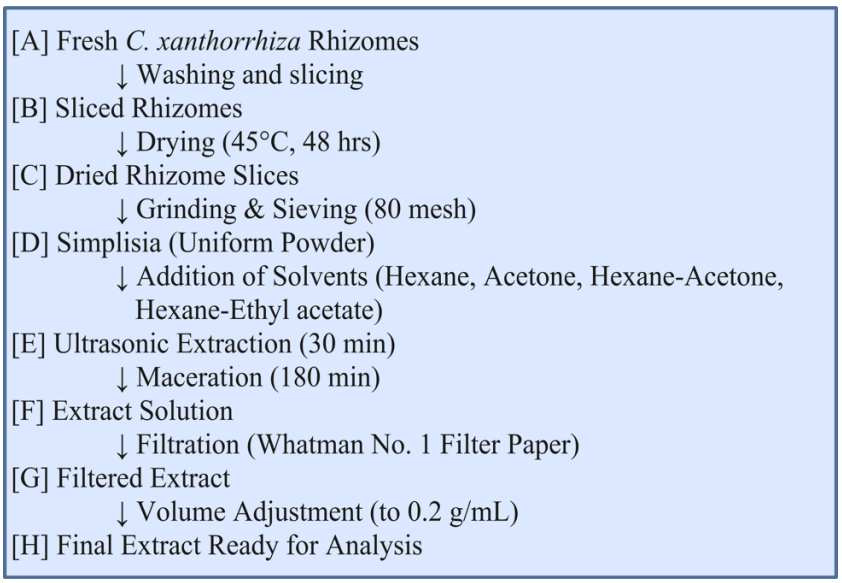

Figure 1. Diagram of the extraction procedure workflow.

2.3. Determination of total terpenoid content.

Total terpenoid content was measured following a modified method of Biswas *et al.* [17]. Two reagents were prepared: (1) perchloric acid and glacial acetic acid (10:3 v/v), and (2) vanillin (0.75 g) dissolved in 15 mL of glacial acetic acid. In a 96-well flat-bottom microplate (Biologix), 100 µL of extract, 80 µL of reagent 1, and 50 µL of reagent 2 were added. The mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 432 nm using a SPECTROstar Nano microplate reader (BMG LABTECH, Germany). Ursolic acid was used as the standard, and results were expressed as mg UA/g DW (ursolic acid equivalents per gram dry weight).

2.4. Antioxidant capacity assays.

Antioxidant activity was evaluated using DPPH, CUPRAC, FRAP, and ABTS assays. Each assay was conducted in triplicate to ensure reproducibility, and Trolox was used as the reference standard. Results were expressed as µmol Trolox equivalents per gram dry weight (µmol TE/g DW).

2.4.1. DPPH Assay

Following the method by Yatma *et al.* [18], 2.5 mg of DPPH was dissolved in 50 mL of acetone to prepare a 125 µM solution. Equal volumes (100 µL) of extract and DPPH solution were mixed in a microplate well and incubated in the dark for 30 minutes. Absorbance was measured at 515 nm.

2.4.2. CUPRAC assay.

The CUPRAC assay was conducted according to Calvindi *et al.* [19], using 10^{-2} M $CuCl_2$, 7.5×10^{-3} M neocuproine, and 1 M ammonium acetate buffer (pH 7). Each well received 50 µL of extract and each reagent, followed by incubation for 30 minutes. Absorbance was measured at 450 nm.

2.4.3. FRAP assay.

The FRAP assay used a reagent mixture of 10 mM TPTZ (in 40 mM HCl), 20 mM $FeCl_3$, and 300 mM acetate buffer (pH 3.6) in a 1:1:10 ratio. In each well, 10 µL of sample was added to 300 µL of FRAP reagent. After 30 minutes of incubation at room temperature, absorbance was measured at 593 nm.

2.4.4. ABTS assay.

The ABTS assay was performed as per Aisyah *et al*. [20]. A 7.7 mM ABTS solution was mixed with 2.4 mM potassium persulfate (2:1) and incubated in the dark for 12–16 hours. The mixture was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. A 20 µL aliquot of each sample was mixed with 180 µL of $ABTS^+$ solution and incubated for 6 minutes before reading absorbance at 734 nm.

2.5. Statistical analysis.

Data were presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test at a 95% confidence level (exact p-values were reported), utilizing IBM SPSS Statistics version 25.

3. Results and Discussion

3.1. Effect of solvent type on total terpenoid content.

The type of solvent used significantly influenced the total terpenoid content extracted from the rhizomes of Curcuma xanthorrhiza. As shown in Table 1, hexane resulted in the highest terpenoid content (243.74 ± 8.13 mg UA/g DW), while ethyl acetate–hexane yielded the lowest (9.34 ± 0.24 mg UA/g DW). The difference in yield was statistically significant ($p = 0.000$). These results confirm that nonpolar solvents, *such as hexane, are more efficient in extracting lipophilic compounds, including* terpenoids [21]. Terpenoids are primarily composed of sesquiterpenes and monoterpenes, which are soluble in nonpolar media [22,23]. Thus, their higher yield in hexane is consistent with their chemical nature. The relatively low extraction efficiency observed with acetone or ethyl acetate, which are more polar, further supports the nonpolar preference of these compounds. These findings highlight the selectivity of nonpolar solvents in targeting terpenoid fractions during phytochemical extraction.

Table 1. Total terpenoid content of *Curcuma xanthorrhiza* rhizome extracts using different solvents.

Solvent	Total terpenoid content (mg UA/g DW)
Hexane	243.74 ± 8.13^a
Acetone	9.40 ± 0.10^b
Hexane–Acetone	10.16 ± 0.33^b
Hexane–Ethyl acetate	9.34 ± 0.24^b

Different superscript letters indicate significant differences at $p < 0.05$.

3.2. Effect of solvent type on antioxidant capacity.

Antioxidant activity was evaluated using four standard in vitro assays: DPPH, CUPRAC, FRAP, and ABTS. The results, presented in Table 2, demonstrate that acetone consistently yielded the highest antioxidant capacity across FRAP (131.25 ± 4.33 µmol TE/g DW), CUPRAC (92.56 ± 2.24 µmol TE/g DW), and ABTS (15.45 ± 0.38 µmol TE/g DW) assays. The only exception was DPPH, where the hexane–acetone mixture showed the highest

value (18.10 ± 0.25 µmol TE/g DW). Statistical tests confirmed significant differences in antioxidant capacities among the solvent systems (p-values < 0.001).

The results reveal that antioxidant activity is more closely associated with the solvent's polarity than with terpenoid content. Acetone, being a polar solvent, facilitates the extraction of hydrophilic antioxidant compounds such as phenolics and flavonoids [24,25]. The poor antioxidant performance of the hexane extract, despite its high terpenoid content, suggests that terpenoids may not be the primary contributors to antioxidant capacity in *C. xanthorrhiza*. This indicates that compounds such as curcumin, xanthorrhizol, and other polyphenols [7]—known to be abundant in *C. xanthorrhiza*—are likely the major contributors to antioxidant activity.

Additionally, differences in antioxidant capacity across the four assays reflect their underlying chemical mechanisms. CUPRAC and FRAP are based on electron transfer (ET), while DPPH and ABTS involve both ET and hydrogen atom transfer (HAT) [26–28]. The higher sensitivity of FRAP and CUPRAC to electron-donating compounds aligns with the observed trend (FRAP > CUPRAC > ABTS > DPPH) [29,30].

Table 2. Antioxidant capacity of *Curcuma xanthorrhiza* rhizome extracts with different solvents and assays.

Solvent	DPPH	CUPRAC	FRAP	ABTS
	(µmol TE/g DW)*			
Hexane	0.01 ± 0.00d	3.02 ± 0.20d	0.50 ± 0.02c	1.42 ± 0.09b
Acetone	1.08 ± 0.03c	92.56 ± 2.24a	131.25 ± 4.33a	15.45 ± 0.38a
Hexane–Acetone	18.10 ± 0.25a	59.62 ± 1.61b	13.00 ± 0.76b	1.12 ± 0.07b
Hexane–Ethyl acetate	2.37 ± 0.02b	29.39 ± 0.32c	4.65 ± 0.07c	1.13 ± 0.07b

Different superscript letters within a column indicate significant differences at $p < 0.05$.

These findings are consistent with prior studies, including those by Yodi et al. [31], who reported that acetone extracts of C. xanthorrhiza exhibited superior antioxidant activity compared to those obtained with ethyl acetate. Further support comes from Izzati *et al*. [32], who demonstrated that ethanol-based extraction combined with UV–Vis–IR spectral fingerprinting and chemometric modeling effectively authenticated *C. xanthorrhiza*, highlighting the importance of solvent choice in maintaining compound specificity during analytical evaluation. A 2021 study also reported that ethanol-extracted *C. xanthorrhiza* exhibited greater antioxidant activity (IC_{50} = 78.30 µg/mL) compared to its nanoparticle form (IC_{50} = 116.51 µg/mL), underscoring the role of extraction mode and solvent polarity in influencing bioactivity [33]. Moreover, Wikara *et al*. [34] validated the use of HPTLC fingerprinting for standardizing the phytochemical profile of *C. xanthorrhiza* extracts, while Yasacaxena *et al*. [35] reviewed various solvent-assisted extraction methods and their impact on the plant's antibacterial efficacy. Collectively, these findings reinforce the critical role of solvent specificity in optimizing the recovery, identification, and functional efficacy of bioactive compounds for pharmacological or nutraceutical applications..

4. Conclusions

This study demonstrated that solvent polarity has a significant influence on the extraction efficiency of terpenoids and antioxidant compounds from *Curcuma xanthorrhiza* rhizomes. Hexane, a nonpolar solvent, yielded the highest terpenoid content (243.74 ± 8.13 mg UA/g DW), while acetone, a polar solvent, consistently exhibited the highest antioxidant capacity, particularly in FRAP (131.25 ± 4.33 µmol TE/g DW) and CUPRAC assays. These findings suggest that terpenoids are not the main contributors to the antioxidant activity of *C. xanthorrhiza*. This highlights the importance of phenolic and flavonoid compounds, which are

more extractable in polar solvents, as key antioxidant agents. Therefore, further investigation is needed to identify other bioactive compounds—such as phenolics or flavonoids—that may play a dominant role in its antioxidant potential. These insights support the rational selection of extraction solvents to align with desired bioactivity targets, providing practical guidance for the formulation of phytopharmaceuticals and nutraceuticals. The results provide a foundation for optimizing solvent selection based on specific phytochemical targets in medicinal plant research and the development of formulations.

Author Contributions

Conceptualization, W.N. and I.M.A.; methodology, S.W.; formal analysis, S.W. and W.N.; investigation, S.W.; resources, W.N.; data curation, S.W. and W.N.; writing—original draft preparation, S.W., I.M.A. and W.N.; writing—review and editing, S.W. and W.N.; supervision, W.N. and I.M.A.. All authors have read and agreed to the published version of the manuscript

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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