Antioxidant activity of Artemisia (Artemisia annua) extract on several concentrations and solvents

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Abstract. Artemisia contains secondary metabolites with antioxidants properties. The study aimed to obtain the type and concentration of effective solvents to produce Artemisia extract with maximum antioxidant activity. The research was conducted at the Laboratory of Indonesian Spices and Medicinal Crops Research Institute in 2015. The Artemisia used for the research came from Lembang, West Java. The study was arranged in a randomized block design, 7 treatments, and 2 replications. The treatment consisted of a combination of two solvents (ethanol and methanol) and three solvent concentrations (50, 70, and 96%) and water as control. Artemisia was processed into powder and then extracted following the treatments. The extract obtained was then tested for its antioxidant activity using the DPPH method. The type and concentration of solvent had a significant effect on the yield and antioxidant activity of Artemisia extract. The use of ethanol solvent resulted in higher extract yield and antioxidant activity than methanol. The higher the solvent concentration, the smaller the extract yield. The antioxidant activity of Artemisia ethanolic extract was stronger than methanolic extract. The IC₅₀ value of the Artemisia ethanolic and methanolic extract were 20.61 ppm, and 25.06 ppm. The most effective solvent concentration to extract Artemisia was ethanol 70%.

Keywords: Artemisia annua, yield, secondary metabolite

1. Introduction

Antioxidants can retard or prevent cell damage caused by free radicals. The antioxidants can come from synthetic and natural sources, but the continuous use of synthetic materials caused adverse effects on health, such as cancer [1]. One of the main compounds in antioxidant activity was the phenolic group [2]. Phenolic compounds were able to neutralize free radicals that were harmful to the body. The activity of phenolic compounds are wide ranging, including antioxidant, antimutagen, anticarcinogenic, and gene expression modification [3, 4, 5]. Natural antioxidants can be sourced from various plants, enzymes, and proteins [6].

Artemisia (Artemisia annua Linn) is a shrub-shaped plant and belongs to the family Asteraceae [7]. Artemisia has long been used in China as a medicinal plant [8]. Artemisia had been widely studied as an antimalarial, antitumor [9], and antioxidant [10]. It contained artemisinin compounds and various components of essential oils [11, 12]. The simplicia contained 0.064% artemisinin [13]. According to Tang & Eisenbrand [14], the chemical compounds contained in Artemisia belong to the sesquiterpene group (artemisinic acid, artesiamut acid, and qinghao acid) and the flavonoid group (coumarin, scopoletin, artemetin, esculetin tetramethoxy flavones). Moreover, Artemisia extract contained flavonoids, terpenoids, fatty acids, coumarins, phenols, artemisinic acids, and essential oils [15] and...
Artemisia's active compound was soluble in semi-polar solvents, such as acetonitrile which was very expensive. Therefore, it is necessary to find a cheaper solvent. Methanol solvent, which has a polarity similar to acetonitrile, can be used to extract Artemisia. Methanol and water were effective solvents for extracting phenolic components found in natural materials [17].

Extraction effectiveness was influenced by various factors such as temperature, extraction time, type and concentration of solvent, the ratio of material to solvent, and particle size [18]. In addition, different solvent concentrations showed different polarities, which affected the flavonoids solubility [19]. Therefore, it is necessary to determine the suitable type and concentration of the solvent in the extraction process to obtain maximum levels of bioactive components and antioxidant activity.

This study aimed to obtain the type and concentration of solvents that effectively extracted the bioactive compounds in Artemisia to produce a high yield of artemisia extracts with strong antioxidant activity.

2. Materials and methods

2.1. Research design

The study was arranged in a randomized block design, 7 treatments, and two replications. The treatments were type and concentration of the solvent: water (control), 50% methanol, 70% methanol, 96% methanol, 50% ethanol, 70% ethanol, and 96% methanol. Parameters observed were extract yield and antioxidant activity (IC_{50}).

2.2. Simplicia preparation

Artemisia leaf was obtained from Manoko (Lembang), West Java, Indonesia. The leaves were washed with tap water, drained and sun-dried covered with a black cloth to prevent bioactive damage. The water content was determined by the crunchiness of the leaves. Leaves were considered to have appropriate water content if they can be crushed handily. The maximum of water content for simplicia is 10%.

2.3. Artemisia extraction

Artemisia simplicial was ground into powder using a mill and then sieved to obtain artemisia powder with the size of 50-60 mesh. The solvent was added following concentration treatments into 250 g of artemisia powder with a ratio of 1:5. The solution was then mixed and stirred for 4 hours and extracted using the maceration method for 24 hours. The next stage was filtering the extraction results with filter paper onto a glass funnel to separate the filtrate from the residues. The filtrate was evaporated using a rotary evaporator to separate the extract from the solvent to get a thick extract. The extract yield was calculated using the formula below

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\text{Extract yield (\%) = \frac{\text{amount of extract yield (g)}}{\text{amount of extract material}}} \times 100
\]

2.4. Antioxidant activity analysis

Artemisia extracts obtained from various treatments were tested for their antioxidant activity using the DPPH method (2,2-difenil-1-pikrilhidrazil) [20]. The extract was pipetted as much as 0.5; 1; 1.5 ; 2; and 2.5 ml, then diluted with methanol + water (1:1) in a 10 ml volumetric flask to get the concentrations of 50, 100, 150, 200, and 250 g/ml respectively. The 1 ml of sample solution was put into a test tube and was mixed with 1 ml of 100 g/ml DPPH solution and 2 ml methanol. The mixture was homogenized and placed in the darkroom for 30 minutes. The solution absorption was measured by UV-Vis spectrophotometer at a wavelength of 515.5 nm with gallic acid as a comparison. The effectiveness of antioxidants was determined by the IC_{50} (Inhibition concentration 50%) value, which was a value that indicated the amount of tested extracts that can capture free radicals by 50%. The IC_{50} value was indicated by the linear regression line equation which represented the correlation between the concentration of the sample compound (X) and the average radical scavenging activity/inhibition (Y). Thus, the IC_{50} value described the concentration of the extract that can scavenge free radicals by
50% through a linear regression equation that exhibited the correlation between the concentration of the test compound (sample) (X) and the average radical scavenging activity (Y) of the measurement replication series.

3. Results and discussion

3.1. Yield extract

The yield extract of Artemisia using the maceration method ranged from 16.48 to 30.52% (Figure 1). The highest yield was at 50% methanol solvent treatment, although it was not significantly different from 50% ethanol solvent. In contrast, the smallest yield extract was at 96% ethanol solvent, whereas extraction with water solvent produced 34.76% yield extract. Likewise, at 70% solvent concentration, there were no significant differences between methanol and ethanol solvents. The significant difference between methanol and ethanol was only showed at a concentration of 96% (Figure 1).

The type and concentration of the solvent had a significant effect on the yield of artemisia extract. Methanol solvent produced a higher yield than ethanol solvent. The higher the solvent concentration, the lower the extract yield produced. This result was following [21]. Ethanol and methanol solvents were more volatile than water.

Moreover, the extraction solvents at high concentrations will evaporate more quickly than low ones due to their boiling point. A low concentration solvent had a high boiling point, hence evaporated slowly resulted in higher extract yield. Solvent concentration at 50% was evaporated slower than 70% and 96% because it contained a certain amount of water. The remaining solvent contained in the extract, especially water, can be evaporated optimally by increasing the temperature of the rotary evaporator. However, this was not recommended because the bioactive compounds contained in the plant can be damaged due to heat sensitivity.

The level of solvent polarity was capable of affecting the extraction process of bioactive compounds. Polar solvents will dissolve polar compounds, and nonpolar solvents will dissolve nonpolar compounds [22]. The effectiveness of the compound extraction by a solvent was highly dependent on the substance solubility in a solvent. The type of solvent in the extraction process affected the content of bioactive compounds produced in the extract [23]. Ethanol, methanol, acetone, and water were all polar [22]. The higher the solvent concentration, the lower the polarity level. Water was the most polar solvent with an electric constant value of 80 [24]. The higher the solvent polarity extracting Artemisia, the higher the extract yield. The solvent with a concentration of 50% produced a

![Figure 1. Extract yield of Artemisia at several types and concentration of the solvents.](image-url)
higher extract yield than 70% and 96%. The water solvent used to extract Artemisia produced the highest extract yield than ethanol and methanol solvents (Figure 1).

The ethanol solvent and water combination was the best extracting solvent for almost all bioactive compounds, with low molecular weight [25]. The type of solvent affects the amount of active compound extracted into the solvent, where polar compounds will dissolve in polar solvents, and nonpolar compounds will dissolve in nonpolar solvents.

The artemisinin belonged to relatively nonpolar sesquiterpene groups [26]. Methanol was a solvent that can dissolve almost all organic compounds, both polar and nonpolar, because methanol has a polar group (-OH) and a nonpolar group (-CH3). Ethanol and methanol solvents had the same level of polarity but had different dielectric constant values. The dielectric constant of ethanol was 33, and methanol was 30 [27]. The type of solvent was an essential factor in determining extraction efficiency. This solvent type was due to the different polarity of antioxidant components [28]. The substance solubility in a solvent was primarily determined by the compatibility between the chemical structure of the solute and the solvent, termed as "like dissolving like" [29]. Solvent concentration in Artemisia extraction had a different effect on the extract yield because the polarity of the solvent was different. According to [19] solvent concentration can affect the solubility of flavonoids found in artemisia extract.

3.2. Antioxidant activity (IC50)

The level of antioxidant activity of artemisia extract was determined by the IC50 value, ranging from 20.61 to 62.00 ppm and 100.8 ppm for the aqueous extract (Table 1). The smallest IC50 value was 20.61 ppm in 70% ethanolic extract, and the highest was 62 ppm in 50% methanolic extract. Artemisia extracted with ethanol solvent had higher antioxidant activity than methanol and water, indicating a smaller IC50 value. The smaller the IC50 value of the extract, the stronger the antioxidant activity [20]. The antioxidant activity was categorized as strong if the IC50 value was <50 ppm, 50-100 ppm (moderate), and 100-200 (weak) [30]. In general, ethanolic and methanolic extracts of Artemisia were categorized as having strong antioxidant activity, except for 50% methanolic extract and aqueous extract, which possessed moderate antioxidant activity (Figure 2).

![Figure 2. The IC50 value of artemisia extract at several types and concentration of the solvents.](image-url)

The solvent's type and concentration affected the artemisia extract's antioxidant activity, as indicated by the IC50 value. The 70% ethanolic extract had stronger antioxidant activity than the 70% methanolic extract, indicating a lower IC50 value. The more bioactive compounds extracted, the
stronger the antioxidant activity of the extract [31]. Ethanolic extract of Artemisia reduced free radicals by 50% at 20.61 ppm, while the methanolic extract was 25.06 ppm (Figure 2).

The type and concentration of a solvent had a significant effect (P>0.05) on the IC₅₀ value and, at the same time, affected inhibition capability (Figure 3, 4, 5). Artemisia ethanolic extract was stronger in reducing free radicals than methanolic extract. Moreover, the active compounds extracted by the 70% ethanol solvent were higher than the methanol solvent (Figure 4).

The highest percentage of inhibition of free radicals was found in the 70% ethanolic and methanolic extract, whereas the ethanolic extract possessed stronger inhibition than methanolic extract (Figure 3, 4). A 20 ppm of 50% ethanolic and methanolic Artemisia extracts could inhibit free radicals by 33.33% and 27.48%, respectively (Figure 3). At 70% concentration, the inhibition percentage was 50.89% and 40.32% for ethanolic and methanolic extract, respectively (Figure 4). Meanwhile, at 96% concentration, the ethanolic and methanolic extract could inhibit free radical 40.67% and 27.48%, respectively (Figure 5). In contrast, aqueous extract (as control) had only 13.74% of inhibitory percentage (data was not shown).

![Figure 3. The correlation between concentration and free radical inhibition (% inhibition) of 50% ethanolic and methanolic artemisia extract](image1)

![Figure 4. The correlation between concentration and free radical inhibition (% inhibition) of 70% ethanolic and methanolic artemisia extract](image2)

![Figure 5. The correlation between concentration and free radical inhibition (% inhibition) of 96% ethanolic and methanolic artemisia extract](image3)
Antioxidant activity is the ability of antioxidants to inhibit oxidation reactions expressed as percent inhibition. The strength of antioxidant activity was influenced by the content of phenolic compounds and total flavonoids [32, 33, 34].

The type and concentration of solvent affect the absorption of Artemisia extract. In addition, the extract concentration used also has an effect. The higher the amount of extract used, the higher the antioxidant inhibition against free radicals. Moreover, the solvent concentration affected the percentage of free radical inhibition. The higher the solvent concentration, the higher the inhibition percentage of the extract against free radicals. However, there might be a limit in solvent concentration because, at 96% concentration, the inhibition percentage was declined (Figure 5).

The bioactive compounds of Artemisia, which hold antioxidant property, had good solubility when extracted with 70% ethanol as solvent. Moreover, the solvent and the polarity level used in the extraction process can affect the amount of bioactive compounds extracted, affecting the strength of antioxidant activity. [35] Also suggested that Artemisia be extracted with ethanol mixed with water as solvent.

4. Conclusion
The type and concentration of solvent significantly affected the yield and antioxidant activity (IC50) of artemisia extract. Artemisia simplicia extracted with methanol produced a higher extract yield than ethanol. However, artemisia ethanolic extract has stronger antioxidant activity than methanolic extract. Therefore, Artemisia was effectively extracted using ethanol at a concentration of 70% to get a high extract yield and strong antioxidant activity.

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Contributorship
All authors have an equal role as the main contributor.

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