Total Phenolic Content, Antioxidant, and Sunscreen Activities of *Daemonorops draco* Resin Extracts from Extraction at Various Ethanol Concentrations and Resin-Solvent Ratio

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Abstract. This research aimed to determine the yield, total phenolic content (TPC), in vitro sunscreen (SPF), and antioxidant activities (AA) of *Daemonorops draco* resin (DDR). The DDR was macerated with ethanol at various concentration (EC) namely 100% (E100), 90% (E90), 80% (E80), and 70% (E70) and the DDR:solvent ratio (D:S) were 1:5, 1:10, and 1:15. This research showed that the interaction between the EC and D:S affected AA, SPF, and TPC of DDR extract (DDRE), but it didn’t affect the DDRE yield. The DDR extraction with E70, E100, and D:S of 1:15 produced the highest DDRE yield. The extraction with E70 and three different D:S produced DDRE with the highest TPC. The highest AA of DDRE was obtained from extraction with E80 and D:S 1:10 (E80-10), E70-15, and E90-5 respectively. The highest SPF of DDRE was obtained from the extraction with E90-15, E70-15, E90-5, and E70-10, respectively. The extraction with E70 and D:S of 1:15 is the best extraction condition because it produces DDRE with a yield of 17.4±3.9%-20.71±1.6%; TPC of 263.93±5.25 mg GAE/g DDRS; AA with an IC value of 78.3±0.3 ppm, and sunscreen with an SPF value of 33.1±0.0.

1. Introduction

Antiaging cosmetic preparations are currently one of the most popular products and are in demand by the public. This is indicated by the increasing demand for these products in the Indonesian market [1]. The cosmetics industry in Indonesia continues to increase, in 2017 growth reached 6.35%, and in 2018 growth rose to 7.36% and will be targeted to continue to increase to reach 9% in this year. It is known that 60% of consumers in Indonesia want an instant change of beauty products and at the same time, products beauty must contain antiaging ingredients nature, especially plants [2]. Antiaging cosmetic preparations must contain antioxidant active ingredients, sunscreen, anti-inflammatory, maintain skin elasticity rejuvenation, and skin lightening [3]. Currently, anti-aging cosmetic preparations on the market still use expensive imported active ingredients such as synthetic materials which are dangerous if used in the long term [3]. Examples are synthetic antioxidant active ingredients such as butyl hydroxyl anisole (BHA) and butyl hydroxytoluene (BHT) which are carcinogenic [4]. Synthetic sunscreen active ingredients such as oxybenzone, avobenzone, p-aminobenzoic acid (PABA), TiO2, and ZnO derivatives that are used in the long-term cause allergic effects, hypersensitivity, inhibition of vitamin D synthesis, and the accumulation of these ingredients can also increase the risk of melanoma cancer. [2]. For this reason, it is necessary to explore natural antioxidant compounds and sunscreens as substitutes for active ingredients in synthetic antiaging cosmetic preparations.
Daemonorops draco resin (DDR) is a non-timber forest product that can be used as a source of antioxidants and natural sunscreens. There are three compounds in DDR as dracohordin, 3,4-dihydro-5-methoxy-6-methyl-2-phenyl-2H-1-benzo-pyran-7-ol, and trendione which act as natural antioxidants [5]. DDR contains triterpene compounds, flavans, chalcone [6], diterpenes acids [7], bioflavonoids [8], and anthocyanins or proanthocyanidins which are used as active ingredients in topical cosmetic formulations [9], [10]. The antioxidant activity of 100% DDR ethyl acetate extract was strong (IC$_{50}$ 27.61 g/mL). However, in its application, ethyl acetate is not suitable as an extraction solvent because it can cause dry and cracked skin and its vapors and liquids can be irritating compared to ethanol solvents [11]. In addition, BPOM recommends ethanol, water, and water-ethanol mixtures as safe solvents for extraction [12]. Previous research reported that DDR ethanol extract had higher antioxidant properties against the free radical 2,2'-diphenylpicryl hydrazyl (DPPH) (antioxidant value 107.87±1.51 and CUPRAC 1181.79±0.64 mol trolox/g). DDR ethanol extract also has a high sunscreen with a sun protection factor (SPF) value that is classified as ultra (SPF 17 value) [13].

Extraction conditions greatly affect the type and composition of extractive substances that cause differences in extract bioactivity. The type of solvent and the ratio of the material and solvent in the extraction process are very important in the diffusion process which affects the chemical compounds extracted [14]. This is confirmed by research [15] which reported that the concentration of ethanol affects the yield, antioxidant activity, and sunscreen of Gyrinops versteegii leaf extract. In a previous study, [13] reported that DDR extraction was carried out until the filtrate was colourless (14 x extraction). This will affect the production cost of the extract when it is commercialized. Extraction optimization is carried out to obtain the most optimal conditions in the extraction process. According to [16], various problems in construction design, extraction, and chemical analysis can be solved by optimization. The objective of the optimization solution is the proper and cost-effective selection of the best variables among the overall and efficient quantitative method processes. Many factors can affect the extraction process such as variations in time, variations in solvent concentration, and the comparison of the value of the extraction raw material with the solvent used.

Several factors in the extraction process affect the extraction results including the type of solvent, the ratio of the weight of the material to the volume of the solvent, temperature, stirring, extraction time, and sample size [17]. According to [18], ethanol has a good ability to extract phenolic compounds from seaweed and terrestrial plants. Compared to methanol or acetone, ethanol is preferred because it is food grade and pharmaceutical grade. According to [19], the content of the phenolic compound phlorotannin and its antioxidant activity from brown seaweed Sargassum serratum is strongly influenced by extraction conditions such as the type of solvent and extraction time. Increasing the concentration of ethanol will decrease the polarity of the solvent used, which in turn can increase the ability of the solvent to extract fewer polar compounds [20]. Less polar solvents can cause cell walls that have less polar properties to be degraded so that the active compounds contained in the sample become easier to extract [21]. Several previous studies have shown that the type of solvent affects the effectiveness of the carotenoid extraction process, for example, in pumpkin carotenoid extraction using a single solvent, namely acetone, ethyl acetate, and n-hexane, the best treatment was obtained from the type of solvent n-hexane [22]. According to [23] that the higher the concentration of ethanol used, the less extract produced, which also means that the more concentrated the extract obtained. This is because the ethanol contained in the solvent, the larger the ethanol fraction, the more will evaporate when vaporized with a rotary vacuum evaporator so that the volume of the extract is less, compared to solvents that contain less ethanol composition because the boiling point of ethanol is lower than water. In papaya seed extract using ethanol, acetone, and ethyl acetate as solvents, the best treatment was obtained from the type of solvent that produced the highest total phenol obtained in ethanol solvent [24]. Research [25] is the optimization of the fucoxanthin extraction process of Padina australis Hauck seaweed using polar organic solvents acetone, acetonitrile, dimethyl sulfoxide (DMSO), ethanol, and methanol from Madura waters. The observed response was based on the results obtained by the value of total phenol content, antioxidant activity, and the effectiveness of SPF extracts extracted from DDR. Therefore, this study aims to analyze the effect of ethanol concentration and the comparison of the value of the extraction raw material (DDR) with the solvent used on the total phenol content, antioxidant activity, and sunscreen.
2. Materials and Methods

2.1. Plant Materials

The raw material used in this research is DDR, which is the result of resin extraction from *D. draco* fruit rattan by the Acehnese people. DDR was obtained from the Indonesian Dragon-blood Association.

2.2. Chemicals and Instruments

Chemicals used include DPPH, ethanol, Folin-Ciocalteu solution, gallic acid, and Trolox. The tools used are ELISA plate well reader (Brand Biotek Epoc), and UV-Vis Spectrophotometry.

2.3. Design of Experiments for *D. draco* Extraction

This study used a factorial design, namely the ethanol concentration factor (70, 80, 90, and 100%) and the resin-solvent ratio of 1:5, 1:10, and 1:15. The responses analyzed were extraction yield, total phenol content, antioxidant activity against DPPH free radicals, and sunscreen activity as measured by the SPF value. If the results of the analysis of variance (ANOVA) show that the interaction of the two factors has a significant effect or each factor has a significant effect (if the interaction has no significant effect), then Duncan’s further test is carried out.

2.4 Extraction

Each DDR sample was extracted with ethanol (ethanol concentration 70, 80, 90, and 100%) and the resin-solvent ratio of 1:5, 1:10, and 1:15. Extraction was carried out by the maceration method, which was soaked at room temperature for 24 hours. Extraction was carried out 3 times. Extraction repetitions used in each run order are 3 replications. DDR extracts from ethanol extraction of 70, 80, 90, and 100% at a resin-solvent ratio of 1:5 were shortened to E100-5, E90-5, E80-5, and E70-5, while the resin-solvent ratio 1:10 is shortened to E100-10, E90-10, E80-10, and E70-10. The DDR extract extracted with ethanol solvent and the resin-solvent ratio of 1:15 was shortened to E100-15, E90-15, E80-15, and E70-15. The concentration of the extract in the filtrate was determined by taking 3 x 10 mL of the extraction filtrate from all the resulting filtrate placed in a petri dish and then in an oven at a temperature of 103±2°C to obtain a constant weight of the extract for determination of the yield [26].

2.5 Total Phenolic Content (TPC) Analysis

The Folin-Ciocalteu method used in the determination of total phenolic refers to [27]. 20 μL of extract solution was added with 100 μL of Folin-Ciocalteu reagent and mixed in a test tube and then homogenized for 5 minutes. Then, 80 μL of 7.5% Na₂CO₃ solution was added (30 minutes incubation). After homogenization, the abrasion measurement was carried out using a microplate reader at a wavelength of 750 nm. Determination of total phenolic compounds was expressed by milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

2.6 Antioxidant Activities

The DPPH method used in the determination of antioxidant activity refers to [28]. A total of 100 μL of DPPH free radical solution with a concentration of 125 M in ethanol was added with 100 μL of DDR extract solution in a 96-well plate and incubated for 30 minutes at room temperature. Furthermore, the absorption results will be measured using a microplate reader (Epoch Biotek, USA) at a wavelength of 515 nm. Trolox standard curves were prepared using a concentration range of 1.25-50 mol ET/g extract. The value of antioxidant capacity is expressed in the IC₅₀ value.

2.7 Sun Protector Factor

Determination of the effectiveness of sunscreen refers to [29] and is expressed as SPF. The determination of the SPF value will refer to the Food and Drug Administration (FDA) which is known to classify the effectiveness of sunscreen preparations based on the SPF value. The SPF value was observed using a UV-Vis spectrophotometer with a wavelength of 290-360 nm and ethanol as a standard as a solvent. Absorbance data will be read using 2.5 nm interval length. Testing the effectiveness of sunscreen was carried out 3 times.
3. Result and Discussion

3.1. Yield of Extraction

The yield of DDR extraction ranged from 13.10±1.41% to 22.15±0.54% (w/w). The results of the analysis of variance (ANOVA) showed that the interaction between the ethanol concentration factor and the resin-solvent ratio had no significant effect on the yield of DDR extract. However, each factor affects the yield. Figure 1 shows that increasing the ethanol concentration from 70-90% caused a decrease in the yield of DDR extract. However, at 100% concentration, there was an increase in the yield of DDR extract. This could be because the terpenoid compounds contained in DDR were extracted higher in 100% ethanol compared to ethanol at lower concentrations. According to [30], the terpenoid compounds that make up the resin are easily soluble in non-polar solvents. Ethanol contains a non-polar side so that it can dissolve non-polar compounds even though the solubility is not as large as non-polar solvents. The decrease in the concentration of ethanol will reduce its ability to dissolve the terpenoid compounds that dominate DDR. The yield of E70 extract was higher than E80 and D90 due to the increasing number of phenolic compounds in DDR which were extracted in a more polar ethanol solvent. Duncan's further test results showed that the highest yield of DDR extract was E100, but the yield value was not significantly different from that of E70 extract (Figure 1).

![Figure 1](image.png)

Figure 1. Main effects plot for the yield of DDR extracts. The different letters on the graph indicate a significant difference at the 95% confidence interval.

Figure 1 shows that the higher the amount of solvent used for extraction, the higher yield produced. Duncan's further test results showed that the highest yield was obtained from the resin-solvent ratio of 1:15 and the yield value was significantly different from the other resin-solvent ratios. This can happen because the extraction conditions greatly affect the type and composition of the extractive substances causing differences in the bioactivity of the extracts. During the extraction process, the active ingredients will be dissolved by a solvent of suitable polarity. Phenolic compounds are compounds that are polar [31] so it is a necessary polar solvent. [32] explained that ethanol, methanol, and acetone are a type of solvent that is often used for extracting phenolic compounds in plants and herbal plants. During the extraction, process yield will increase along with increasing the amount of solvent. Enhancement of this yield is caused by the higher the amount of solvent used, then the output target compound into the solvent can run more optimal and the solvent is saturated can also be avoided. However, after the number of the solvent is increased by a certain amount then the increase in yield is relatively small and tends to become constant [33]. The type of solvent and the ratio of materials and solvents in the extraction process are very important in the diffusion process which affects the chemical compounds extracted [34]. This is confirmed by a study [35] that reported that the concentration of ethanol affects the yield, antioxidant activity, and sunscreen of Gyrinops versteegii leaf extract. Phenolic compounds are the
largest group of compounds that act as natural antioxidants in plants, one of which is DDR plants. Phenolic compounds have one (phenol) or more (polyphenol) phenol rings, namely hydroxy groups attached to aromatic rings so that they are easily oxidized by donating hydrogen atoms to free radicals. Its ability to form stable phenoxy radicals in oxidation reactions causes phenolic compounds to be very potential as antioxidants. Natural phenolic compounds are generally in the form of polyphenols that form ether, ester, or glycoside compounds, including flavonoids, tannins, tocopherols, coumarins, lignins, cinnamic acid derivatives, and polyfunctional organic acids [33].

3.2. Total Phenolic Content

The TPC of DDR extracts ranged from 124.54±9.09 to 263.93±5.25 mg GAE/g extract. The ANOVA results showed that the interaction between the ethanol concentration factor and the resin-solvent ratio had a significant effect on the TPC of DDR extract. Duncan's further test results on three DDR extracts with the highest TPC values showed that the E70-5 extract was not significantly different from E70-10 and E70-15. However, all three contain higher TPC than other extracts (Figure 2).

![Ethanol Conc * Ratio Solvent](image)

**Figure 2.** Interaction plot for TPC of DDR extracts. The different letters on the graph indicate a significant difference at the 95% confidence interval.

Figure 2 shows that the DDR extract resin-solvent ratio of 1:10 has a significantly higher TPC value than the DDR extract ratios of 1:5 and 1:15 (Figure. 2). This happens because 70% ethanol in resin-solvent ratios 1:5, 1:10, and 1:15 can extract more phenolic compounds than other solvents, especially extracts extracted with 100% ethanol solvent at resin-solvent ratio 1:15 which contains the lowest TPC. Polar phenolic compounds are more soluble in more polar solvents [34]. This can confirm that 100% ethanol even though the extraction yield is higher (Figure 1) there is a tendency to contain lower TPC compared to ethanol with lower concentrations (Figure 2). Differences in total phenol content can occur because it depends on the material and its solubility in various polarities of the extraction solvent [35].

3.3 Antioxidant Activities

The antioxidant activity of DDR extract is indicated by the lower the IC₅₀ value, the higher the antioxidant value. The IC₅₀ value of DDR extract ranged from 43.68±0.11 to 173.32±1.05 ppm. The ANOVA results showed that the interaction between the ethanol concentration factor and the resin-solvent ratio had a significant effect on the antioxidant activity of the DDR extract. Duncan's further test results showed that E80-10 extract had the highest antioxidant activity and was different from other extracts. The extracts with lower antioxidant activity than the E80-10 extracts were the E70-15 and E90-5 extract groups, and the E70-5 and E100-5 extract groups, respectively (Figure 3).
Figure 3. Interaction plot for antioxidant of DDR extracts. The different letters on the graph indicate a significant difference at the 95% confidence interval.

Figure 3 shows that the E80-10 extract had higher antioxidant activity than E100. This was positively correlated with the total phenol content compared to extracts of E100%, E90%, and E70% (Figure 2). This could be due to the antioxidant activity influenced by the total phenol content. This is in line with research [36] that the strongest positive correlation between total phenolics and antioxidant activity of microalgae. Total phenol content can be an indicator of effectiveness as a free radical scavenger. This is because flavonoids from the phenolic compound group can produce phenoxyl radicals which are stabilized by the resonance effect of the aromatic ring [37].

Figure 4 shows the mechanism of flavonoids as free radical scavenger. The purity of a sample during the extraction process can affect the antioxidant activity of the sample [38]. According to [39], the presence of protein and fat in the extract can interfere with the process of scavenging free radicals by phenolic compounds or flavonoids because proteins or fats in plants can donate their hydrogen atoms so that they will bind to hydroxyl radicals in DPPH.

Figure 4. Mechanism of flavonoid as free radical scavenger, a): structure of flavonoids, b): Scavenging process of free radical by flavonoids [40].
3.4 Sun Protector Factor

The sunscreen activity of DDR extract was indicated by the higher SPF value. The SPF value of the DDR extract ranged from 8.06±0.03 to 35.29±0.08. The ANOVA results showed that the interaction between the ethanol concentration factor and the resin-solvent ratio had a significant effect on the sunscreen activity of DDR extracts. Duncan’s further test results showed that the extract E90-15 had the highest SPF value and was followed by extracts E70-15, E90-5, and E70-10 which statistically showed significantly different values from one another (Figure 5).

Figure 5. Interaction plot for SPF value of DDR extracts. The different letters on the graph indicate a significant difference at the 95% confidence interval.

Figure 5 shows that increasing the ethanol concentration from 70-90% with a resin-solvent ratio of 1:5 causes an increase in the SPF value of the DDR extract. This is inversely proportional to the TPC value (Figure 2). This can happen because the concentration of E70-5 with a TPC higher than E80-5, E90-5, and E100-5 is thought to be due to containing lower flavonoid aglycones than other extracts. After all, the polarity of flavonoid aglycones is lower than that of flavonoid glycosides or flavonoid glycosides. simple phenolic compounds so that their solubility in E70 is lower than in E80, E90, and E100. Flavanones play a very important role as protection against UV rays. They are flavonoids, which have absorption bands of 275-295 nm and 300-330 nm [41]. The SPF value of the E100-5 extract was lower than E90-5 and E-8-5. This is because the E100-5 extract contains more terpenoid groups. Figure 5 shows that DDR ethanol extract has the potential to be used as an active sunscreen ingredient. The results of the SPF calculation show that the ethanol extract of DDR belongs to the ultra-protection category. According to Wilkinson and Moore [42], the requirement for the active ingredient to be used as sunscreen is that it is effective in absorbing arrhythmogenic rays in the wavelength range of 290-320 nm and does not cause toxicity. In addition, the active ingredient must provide full transmission in the 300-400 nm wavelength range to provide maximum tanning effect. Sunscreens can absorb at least 85% of sunlight at a wavelength of 290-320 nm for UVB but can transmit light at a wavelength of more than 320 nm for UVA. Therefore, sunscreen is needed that can protect the skin from the dangers of solar radiation. According to [43], antioxidants in sunscreen preparations can increase the photoprotective effectiveness of substances that are antioxidants and prevent various diseases caused by UV radiation.

4. Conclusion

The interaction between the ethanol concentration and the resin-solvent ratio affected antioxidant activity, sunscreen, and TPC values, but the interaction did not affect the yield of DDR extract. The highest yield of DDR extract was DDR extract of E100 and E70. The higher the amount of solvent used,
the higher the extract yield. The highest TPC values of DDR extracts were the extract group E70-5, E70-10, and E70-15 followed by E80-10 and E90-10. The highest antioxidant value of DDR extract was extract E80-10 (IC_{50} 43.68±0.11 ppm) followed by extract groups E70-15 and E90-5). The highest value of sunscreen activity of DDR extract was E90-15, followed by E70-15, and E70-10 respectively. Based on yield, TPC, antioxidant activity, and sunscreen, the extraction condition to produce a prospective DDR extract for further development is the extraction with ethanol concentration of 70% and a resin-solvent ratio of 1:15.

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