

## Article

# Antioxidant Activity, Sun Protection Activity, and Phytochemical Profile of Ethanolic Extracts of *Daemonorops acehensis* Resin and Its Phytosomes

Rita Kartika Sari <sup>1,2,\*</sup>, Yanico Hadi Prayogo <sup>1</sup>, Salman Arib Rozan <sup>1</sup>, Mohamad Rafi <sup>2,3</sup> and Ietje Wientarsih <sup>2,4</sup>

<sup>1</sup> Department of Forest Products, Faculty of Forestry and Environment, IPB University, Bogor 16680, Indonesia; yanicohadi@apps.ipb.ac.id (Y.H.P.); salmanaribrozan@gmail.com (S.A.R.)

<sup>2</sup> Tropical Biopharmaca Research Center, IPB University, Bogor 16128, Indonesia; mra@apps.ipb.ac.id (M.R.); ietjewientarsih@gmail.com (I.W.)

<sup>3</sup> Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia

<sup>4</sup> Department of Clinic, Reproduction and Pathology, IPB University, Bogor 16680, Indonesia

\* Correspondence: rita\_kartikasari@apps.ipb.ac.id

**Abstract:** *Daemonorops* (Indonesian: *jernang*) resin is one of Indonesia's leading non-timber forest products and can be developed as a source of natural antioxidants and sun protection. This study aimed to select promising solvents for extracting a *Daemonorops acehensis* resin and phytosome formulation with high antioxidant capacities and sun protection factor (SPF) values. *Jernang* resin was extracted using a water–ethanol mixture in five different ratios. The promising extract was then mixed with soy lecithin in three different formulations. A promising extract and phytosome were then selected based on their antioxidant capacities and sun protection factor (SPF) values. A liquid chromatography mass spectrometry/mass spectrometry (LC–MS/MS) analysis was also performed on five extracts to identify the components in the extracts that might be responsible for the biological activity. The results showed that the ethanol solvent variation and phytosome formulation influenced the antioxidant capacity and SPF value. A hundred-percent ethanolic extract and F1 phytosome exhibited the highest antioxidant capacities and SPF values. A qualitative analysis revealed the various classes of compounds in the extract and phytosome. A flavylum chromophore, dracorhodin, dominated the resin extract and was presumed to be the marker compound responsible for their antioxidant capabilities and SPF values. These findings are important for manufacturing sunscreens containing active compounds of bioactive natural resins.

**Keywords:** antioxidant capacity; *jernang*; SPF value; sunscreen



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## 1. Introduction

Reactive oxygen species (ROS) are dangerous components in cell metabolism because they cause oxidative stress that can damage cells in uncontrolled amounts [1]. Excess ROS has been associated with many degenerative diseases that are also related to the skin's aging process. The skin's aging process is related to UV radiation, which can increase ROS production so that a process occurs that triggers photoaging [2]. Antioxidants are components that are known to have a function in overcoming ROS [3], and many antioxidant agents come from natural resources. Moreover, several natural compounds have been developed as sunscreen agents [4]. Furthermore, there is great potential for exploring various other sources of natural raw materials as sources of antioxidants and sunscreen agents.

Dragon's blood, a red resin secreted from *Daemonorops* fruits (Indonesian: *jernang*), is Indonesia's leading non-timber forest product. *Jernang* resin from the *Daemonorops*

species is only found in Indonesia and Malaysia. The *Daemonorops* species known as a producer of *jernang* resin from Indonesia is *Daemonorops draco* (Willd.) Blume, whereas in Indonesia, there are 115 species of *Daemonorops*, of which 12 species produce *jernang* resin. One of them is *D. acehensis* Rustiami [5]. This species is easily found in the Aceh province (on the island of Sumatra), especially in the districts of Meulaboh, Lhokseumawe, and Tapaktuan. There is no specific information regarding the chemical components of *D. acehensis* resin. However, the previous report showed the main chemical components of dragon's blood from the *Daemonorops* tree, namely *D. draco* and *D. propinqua* resin, are flavylum compounds, such as dracorhodin [6]. Moreover, the previous phytochemical studies regarding *D. draco* resin led to the isolation of various flavonoids [7,8]. The similarity of chemical the profiles of *D. draco* and *D. propinqua* resins also shows the potential similarity of compound components in *D. acehensis* resins. Moreover, a previous report showed that flavonoids had good antioxidant agents and photo protectors [9,10]. It also shows that the flavonoid content in *Daemonorops* resin has potential as an antioxidant and photoprotector.

In a previous report using *D. draco* resin, the antioxidant activity of the ethyl acetate extract was relatively high, with a half-maximal inhibitory concentration (IC<sub>50</sub>) of 27.61 µg/mL [11]. Moreover, the methanol extract of *D. draco* resin from Sarolangun (Jambi, Indonesia) has antioxidant content with an IC<sub>50</sub> value of 117.63 ± 3.02 µg/mL [12]. However, there is no report regarding the antioxidant activity of *D. acehensis*. This also showed potential in developing *D. acehensis* resin as an antioxidant and sunscreen agent since a previous report revealed a relationship between antioxidants and sun protection activity [13]. However, in its application, *D. acehensis* resin extracted with ethanol is safer than that extracted with methanol and ethyl acetate because the ethyl acetate and methanol can cause dry and cracked skin. Its vapors and liquids can be irritating compared with ethanol solvents [14]. The extraction of *D. draco* resin with 80% ethanol solvent and a resin-solvent ratio of 1:10 resulted in an extract with the highest antioxidant activity (IC<sub>50</sub> 43.68 ± 0.11 µg/mL), and it has a sun protection factor (SPF) value of 33.1 [15]. Therefore, evaluating the antioxidant activities and sun protection abilities of extracts extracted using ethanol at various concentrations is necessary.

On the other hand, the high polarity of ethanol-soluble phytoconstituents causes low bioavailability because penetrating cell membranes is challenging [16,17]. Thus, modifying the polarity of the extract by changing its phytoconstituents using the phytosome method is necessary so that the body can effectively absorb the extract. Phytosomes are used to incorporate plant extracts or polar phytoconstituents into phospholipids to produce lipid-compatible molecular complexes. The phytosome form has been used to enhance its bioavailability, remarkably increasing the release of compounds in the gut, maintaining bioactivity in long-term storage, or preventing degradation and increasing penetration in the skin [17–19]. In the manufacture of phytosomes, lecithin derived from soybeans was used. Soy lecithin was chosen as an additive in phytosomes because it is less carcinogenic and flows more quickly in the membrane than pure lecithin and egg lecithin [20]. In addition, a previous report demonstrated increased absorption and penetration of boswellic acids formulated with soy lecithin (phytosome form) [21]. Therefore, this research aimed to select promising extracts with different compositions of the ethanol solvent based on their antioxidant capacity and SPF value. The promising extract was used to make the phytosome complex and to choose the best phytosome formula with excellent bioactivity to increase its bioavailability.

## 2. Materials and Methods

### 2.1. Materials

The *jernang* resin was extracted from the *Daemonorops acehensis* fruit, which was obtained from Aceh (Indonesia) through the Indonesian Dragon's Blood Association. This plant species was confirmed by Herbarium Bogoriense, the Indonesian Institute of Life Science with the letter number of B-909/V/DI.05.07/12/2021. The chemicals are ethanol; dichloromethane; hexane; soy lecithin; purified water; 2,2-diphenyl-1-picrylhydrazyl

(DPPH);  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , ammonium acetate;  $\text{K}_2\text{S}_2\text{O}_8$ , 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); Trolox; dimethylsulfoxide; methanol; polytetrafluoroethylene membrane; formic acid; and acetonitrile from Merck (Massachusetts, MA, USA).

## 2.2. Extraction

Ten grams of *D. acehensis* resin powder was extracted with 100 mL of solvent using the maceration method (immersed and soaked at room temperature). Immersion was carried out using a mixture of ethanol–water with ratios of 1:0 (E100), 3:1 (E75), 2:2 (E50), 1:3 (E25), and 0:1 (E0) for 24 h at ambient temperature. The filtrate was filtered; then, the immersion was repeated fourteen times until a colorless filtrate was obtained. The filtrate was concentrated using a rotary vacuum evaporator and dried in an oven at 50 °C. The dry extract obtained was weighed, and the extraction yield was determined.

## 2.3. Antioxidant Capacity Assay

In the DPPH method [22], 120  $\mu\text{L}$  of the 125  $\mu\text{mol/L}$  DPPH solution was inserted into a 96-well microplate that contained 40  $\mu\text{L}$  of the extract and phytosomes. The solution was incubated for 30 min at room temperature. The absorbance was measured at a wavelength of 515 nm using a microplate reader (Epoch Biotek, Winooski, VT, USA). Trolox was used as a positive control. The measurements were carried out with three replications. The antioxidant capacity was expressed in micromoles of Trolox/gram of dried powder.

In the cupric reducing antioxidant capacity (CUPRAC) method [23], a total of 1 mL of extract and phytosomes dissolved in 96% ethanol was added to 1 mL of 0.01 M  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 1 mL of 0.0075 M ethanolic neocuproine, 1 mL of 1 M ammonium acetate buffer at pH 7, and 0.1 mL of distilled water. The solution was allowed to stand for 30 min, and the absorbance was measured at 453.4 nm. Calibration curves were created using Trolox solutions with various concentrations. The antioxidant capacity was expressed in micromoles of Trolox/gram of dried powder.

In the ABTS method [24,25], the ABTS radical was generated by mixing 5 mL of 7 mM ABTS powder with 88  $\mu\text{L}$  of  $\text{K}_2\text{S}_2\text{O}_8$  140 mM, with all reagents solved in aquabidest. Then, this mixture was stored for 16 h in a dark room with ambient temperature. After generating the ABTS radical, the mixture was diluted in aquabidest with the reagent-aquabidest (1:44 *v/v*). The scavenging activity was measured by mixing 20  $\mu\text{L}$  of the sample and 180  $\mu\text{L}$  of the ABTS reagent in a microplate and incubating the mixture at room temperature for 6 min. A Trolox calibration curve was used as a positive control. The antioxidant capacity was reported in micromoles of Trolox/gram of dried powder.

## 2.4. Sun Protection Factor (SPF) Value

The SPF value was measured using a UV–Vis spectrophotometer at a wavelength of 290–360 nm using a test solution concentration at 125 mg/L and ethanol as a blank. The absorption data were read at 2.5 nm intervals. The measurement was repeated three times. SPF values were counted using the following equation [17]:

$$SPF = Cf \times \sum \frac{360 \text{ nm}}{290 \text{ nm}} EE_{\lambda} \times I_{\lambda} \times Abs_{\lambda} \quad (1)$$

where *Cf* is 10 (a constant),  $EE_{\lambda}$  is the erythemogenic effect,  $I_{\lambda}$  is the intensity of the photon, and  $Abs_{\lambda}$  is the absorbance of the samples

## 2.5. Phytosome–Extract Complex Preparation

The phytosomes were made based on the procedure by Singh and Narke (2015) [16]. The *D. acehensis* resin extract with the highest antioxidant and sun protection activities was mixed with soy lecithin with different ratios in a weight/weight ratio. Formulations 1, 2, and 3 (abbreviated as F1, F2, and F3) consisted of an extract–soy lecithin mixture with weight/weight ratios of 1:1, 1:2, and 2:1, respectively. The *D. acehensis* extract and soy lecithin were mixed and put into a round bottom flask and then refluxed with 20 mL of

dichloromethane at 60 °C for 2 h. The mixture was concentrated using a rotary evaporator to 5–10 mL. Then, n-hexane solvents (20 mL) were added carefully with continuous stirring to obtain a precipitate that was filtered and stored for 12 h in a desiccator. The dry precipitate was crushed in a mortar into a powder, then placed in a glass bottle and stored at room temperature. The experimental design of the phytosome extracts was a completely randomized design with different treatment ratios of soybean extract and lecithin.

#### 2.6. Fourier Transform Infrared (FTIR) Profile

The most active extract and phytosome and soy lecithin were analyzed for their FTIR spectra using a Bruker Tensor 37 FTIR spectrophotometer (Bruker, Karlsruhe, Germany) to detect and determine the chemical bonds/functional groups in the extract and phytosomes of the *jernang* resin extract. The absorbances were measured in the wavenumber range of 600–4000  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$  for 32 scans.

#### 2.7. Identification of Metabolites

The components in the most biologically active extract and phytosome complex (highest antioxidant capacity and SPF value) were identified using ultra-high performance liquid chromatography Vanquish Tandem Q Exactive Plus Orbitrap high resolution mass spectrometry. Then, 5 mg of the sample were added to 2% DMSO, dissolved in 5 mL of methanol, and then filtered with a 0.2  $\mu\text{m}$  PTFE membrane. As much as 5  $\mu\text{L}$  of the sample were injected into the instrument. The separation column type in UHPLC was Accucore C18, 100  $\times$  2.1 mm, 1.5  $\mu\text{m}$  (Thermo Fisher Scientific, Waltham, MA, USA) with a column temperature of 30 °C. Chromatographic separation used the gradient elution method, namely 0–3 min (5–25% B), 3–22.5 min (25–55% B), 22.5–25 min (55–95% B), 25–28 min (95%B), and 29–30 min (5% B). The eluents were  $\text{H}_2\text{O}$  + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B) with a flow rate of 0.2 mL/min. The ionization mode was electrospray ionization (3.80 kV, capillary temperature 320 °C) with a resolution of 70,000. Mass fragment readings were carried out with a scan range of 100–1500  $m/z$ . Qualitative analysis of the phytochemical components was also carried out to identify the differences between extract and phytosome. It was done to determine whether the phytosome modification affected the phytochemical components in the phytosome complex. The analysis was carried out by visual observation using specific reagents based on the method developed in Harborne, 1984 [26]. The parameters were the content of alkaloids, phenyl hydroquinone, flavonoids, tannins, saponins, steroids, and triterpenoids.

#### 2.8. Data Analysis

The yield, antioxidant capacity, and SPF value were analyzed using an analysis of variance with a completely randomized trial design using SPSS 25. Duncan's multiple range test also identified the significance value among groups. The analysis aimed to examine the influence of solvent and phytosome formulations on the yield, antioxidant capacity, and SPF value. The different levels of treatment constitute the concentration of ethanol as the extraction solvent, namely E100, E75, E50, E25, and E0, as well as phytosome formulation (F1, F2, and F3). In addition, the Pearson correlation coefficient was also determined to evaluate the correlation among parameters.

### 3. Results

#### 3.1. Yields, Antioxidant Capacity, and SPF Value of *D. Acehensis* Resin Extracts

The analysis of variance showed that the variation in the solvent type (ethanol concentration) had a significant effect ( $\alpha = 0.05$ ) on the extract yield, antioxidant capacity, and SPF value. The Duncan's further test revealed that the yield value of the E100, E75, and E50 extracts were relatively the same and significantly different from those of the E25 and E0 extracts. However, the tendency is for the ratio of ethanol in the extraction solvent to increase the yield of the extract obtained (Table 1). Consistently, the antioxidant capacity of the extract also increased in the extract with a larger ethanol ratio. The E100 extract has the highest and most significant

antioxidant capacity values using the DPPH and ABTS tests compared with the other extracts. A different phenomenon was found in the CUPRAC test, and the E75 extract had the highest antioxidant capacity but was not significantly different from E100. The antioxidant capacity with DPPH and ABTS has the same trend and yield value. However, this was slightly different from the trend in antioxidant capacity with CUPRAC (Table 1).

**Table 1.** The yield of extraction, antioxidant capacity, and SPF value of *D. acehensis* resin extracts.

| Extracts | Yields (%)         | Antioxidant Capacity ( $\mu\text{mol trolox/g}$ ) |                      |                    | SPF Value            |
|----------|--------------------|---|----------------------|--------------------|----------------------|
|          |                    | DPPH  | CUPRAC               | ABTS               |                      |
| E100     | 33.90 $\pm$ 0.92 a | 107.86 $\pm$ 1.51 a                               | 1181.78 $\pm$ 0.78 a | 52.58 $\pm$ 2.12 a | 17.117 $\pm$ 0.003 a |
| E75      | 33.62 $\pm$ 1.21 a | 82.86 $\pm$ 1.33 b                                | 1182.16 $\pm$ 0.21 a | 45.71 $\pm$ 0.50 b | 11.726 $\pm$ 0.002 b |
| E50      | 32.43 $\pm$ 0.70 a | 56.86 $\pm$ 0.23 c                                | 1157.78 $\pm$ 0.57 b | 38.57 $\pm$ 1.44 c | 7.540 $\pm$ 0.002 c  |
| E25      | 25.55 $\pm$ 1.35 b | 54.40 $\pm$ 0.20 d                                | 1159.16 $\pm$ 1.63 b | 37.89 $\pm$ 0.44 c | 7.293 $\pm$ 0.003 d  |
| E0       | 23.52 $\pm$ 1.06 b | 44.93 $\pm$ 0.31 e                                | 1006.16 $\pm$ 1.98 c | 35.29 $\pm$ 0.78 d | 2.296 $\pm$ 0.001 e  |

Different letters indicate a significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.

The SPF values of the five extracts also showed the same trends for the yield and antioxidant capacity. The E100 extract has the highest SPF value compared with other extracts. The trends in SPF values from low to high are E0, E25, E50, E75, and E100. The similarity of this trend can be confirmed through the Pearson correlation value in Table 2. The SPF value has a significant correlation with the antioxidant capacity and yield parameters. In addition, the three antioxidant parameters—DPPH, CUPRAC, and ABTS—also have high correlations. Based on the yield value, antioxidant capacity, and SPF value, the extraction treatment with 100% ethanol or E100 was chosen as the best extract with the highest yield, the best antioxidant activity, and the highest SPF value.

**Table 2.** The Pearson correlation coefficient among yields, antioxidant capacity, and SPF value of *D. acehensis* resin extracts (\*\* $p < 0.001$ , \* $p < 0.01$ , and \* $p < 0.05$ ).

| Parameters | Parameters |          |          |          |          |
|------------|------------|----------|----------|----------|----------|
|            | Yield      | DPPH     | CUPRAC   | ABTS     | SPF      |
| Yield      | 1.00       | 0.77 *** | 0.77 *** | 0.77 *** | 0.81 *** |
| DPPH       | 0.77 ***   | 1.00     | 0.65 **  | 0.99 *** | 0.98 *** |
| CUPRAC     | 0.77 ***   | 0.65 **  | 1.00     | 0.64 *   | 0.79 *** |
| ABTS       | 0.81 ***   | 0.99 *** | 0.64 *   | 1.00     | 0.96 *** |

### 3.2. Yields, Antioxidant Capacity, SPF Value, and FTIR Profile of E100 Phytosomes

Phytosomes were made using three different formulations, producing different antioxidant activities and SPF values, affecting the yield obtained. The treatment of phytosomes with formula F2 resulted in higher yields than other formulations, but the differences in these formulations did not significantly affect the yield (Table 3). The different formulations of phytosomes resulted in phytosomes with different antioxidant activities and had a significant effect. Based on Table 3, phytosome F1 had the highest antioxidant capacity in the three antioxidant test parameters, and all three were significantly different from other extracts. The antioxidant capacity of the three antioxidant parameters has the same trend, sequentially from low to high, namely F2 < F3 < F1.

**Table 3.** Yields, antioxidant capacity, and SPF value of E100 phytosome.

| Phytosomes Formulation | Yield (%)          | Antioxidant Capacity ( $\mu\text{mol trolox/g}$ ) |                     |                    | SPF Value           |
|------------------------|--------------------|---|---------------------|--------------------|---------------------|
|                        |                    | DPPH  | CUPRAC              | ABTS               |                     |
| F1                     | 78.50 $\pm$ 8.85 a | 277.40 $\pm$ 0.58 a                               | 840.16 $\pm$ 1.15 b | 46.44 $\pm$ 1.19 a | 16.04 $\pm$ 0.003 a |
| F2                     | 80.33 $\pm$ 8.50 a | 124.49 $\pm$ 1.24 c                               | 430.41 $\pm$ 0.57 c | 32.36 $\pm$ 0.13 c | 12.69 $\pm$ 0.100 c |
| F3                     | 78.67 $\pm$ 8.74 a | 242.22 $\pm$ 0.57 b                               | 857.04 $\pm$ 0.78 a | 42.85 $\pm$ 0.79 b | 15.36 $\pm$ 0.002 b |

Different letters indicate a significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.

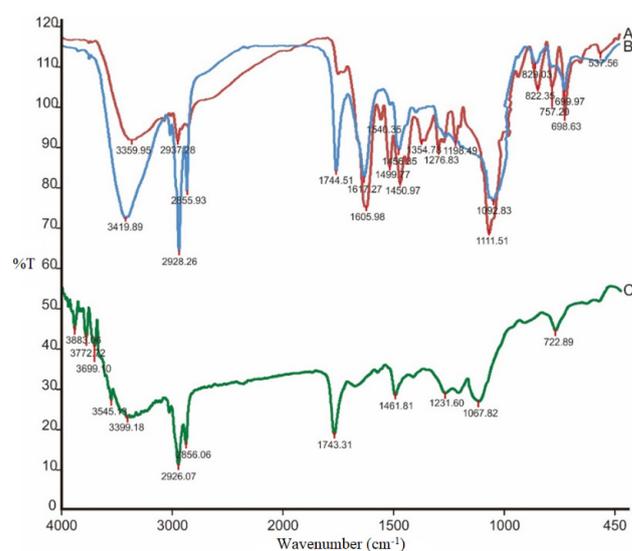
In line with the antioxidant activity, the SPF value also showed the same phenomenon as the antioxidant capacity. Phytosome F1 had the highest SPF value, followed by F3 and F2 (Table 3). Although different in value, the SPF value of F1 was not significantly different from that of F3. The similarity in the trend of SPF and antioxidant capacity was also evidenced by the value of the Pearson correlation coefficient (Table 4). The SPF value and antioxidant capacity have a high and significant correlation coefficient. Different phenomena occur in the yield parameters. The yield of the phytosome formula was not correlated with the antioxidant activity or SPF value.

**Table 4.** The Pearson correlation coefficient among yield, antioxidant capacity, and SPF value of phytosomes (\*\* $p < 0.001$ ).

| Parameters | Parameters |          |          |           |          |
|------------|------------|----------|----------|-----------|----------|
|            | Yield      | DPPH     | CUPRAC   | ABTS      | SPF      |
| Yield      | 1.000      | −0.11    | −0.11    | −0.029    | −0.097   |
| DPPH       | −0.110     | 1.00     | 0.97 *** | 0.990 *** | 1.00 *** |
| CUPRAC     | 0.110      | 0.97 *** | 1.00     | 0.950 *** | 0.97 *** |
| ABTS       | −0.029     | 0.99 *** | 0.95 *** | 1.000     | 0.99 *** |

### 3.3. Phytochemical Profile

The FTIR profile showed a change in the spectral pattern of phytosomes compared with the spectra of *D. acehensis* resin E100 and soy lecithin (Figure 1). The FTIR spectrum of the phytosome showed a combined uptake pattern of E100 and soy lecithin. The absorption peaks at 3419.89, 2928.26, and 2855.93  $\text{cm}^{-1}$ , which are the wavenumber regions for the OH and CH vibrations, are thought to be the implications of the combination of three absorptions of lecithin (3399.18, 2926.07, and 2856.06  $\text{cm}^{-1}$ ) and two absorptions of E100 (3359.98 and 2937.28  $\text{cm}^{-1}$ ). In addition, the carbonyl (C=O) peak was also detected in phytosomes at 1744.51 and 1617.27  $\text{cm}^{-1}$ , which was also detected in the spectrum of E100 and soy lecithin. The typical absorptions of soy lecithin from the P–O–C group and the ester were at 1067.82 and 1231.60  $\text{cm}^{-1}$ .



**Figure 1.** FTIR spectrum of *D. acehensis* resin E100 (A), phytosome (B), and soy lecithin (C).

Meanwhile, the unique absorption of the extract was also in the range of 1100–1300  $\text{cm}^{-1}$ , which comes from C–O (vinyl ether and C–O aliphatic ether). In phytosomes, the absorption peaks of E100 and soy lecithin in the range of 1100–1300  $\text{cm}^{-1}$  overlapped with the main absorption peak at 1092.83 (overlapped C–O and P–O–C). In addition, absorption peaks in the range of aromatic carbons and substituted alkenes were also detected in the extract, soy lecithin, and their complexes (phytosomes). The absorption of cis substituted alkenes

was detected at  $722.89\text{ cm}^{-1}$ , presumably from the unsaturated fatty acid chain from soy lecithin and the absorption of aromatic alkenes at wavenumbers  $698.63$  (meta substituted),  $699.97$  (meta substituted), and  $822.35$  (para-substituted)  $\text{cm}^{-1}$  from compounds in E100. The absorption peak of this substituted alkene also appeared on the phytosome spectrum with absorptions of  $699.97$  and  $829.03\text{ cm}^{-1}$ .

A phytochemical analysis showed that several phytochemical compounds were detected in the extracts and phytosomes. E100 and phytosome F1 extracts with antioxidant activity and the best SPF value contained secondary metabolites, namely alkaloids, phenyl hydroquinone, flavonoids, saponins, and triterpenoids (Table 5). Polyphenolic compounds and steroids were not found in the extracts of E100 and phytosome F1. This qualitative analysis also showed that both samples (E100 and phytosome F1) contained the same group of compounds. This indicates that making phytosomes did not give a qualitative change in the components of the compound.

**Table 5.** Qualitative phytochemical constituent of *D. acehensis* resin E100 and its phytosome.

| Parameter                  | E100 Extract | F1 |
|----------------------------|--------------|----|
| Alkaloid (semi-polar)      | +            | +  |
| Phenylhydroquinone (polar) | +            | +  |
| Flavonoid (polar)          | +            | +  |
| Tannin (polar)             | –            | –  |
| Saponin (nonpolar)         | +            | +  |
| Steroid (nonpolar)         | –            | –  |
| Triterpenoid (nonpolar)    | +            | +  |

An analysis of the compound components showed differences in the composition of the compounds contained in the extract with differences in the composition of the extraction solvent. However, several compounds in the five extracts were not identified. This was due to the limitations of the database in identifying the components of the compound. Table 6 displays 11 compounds that have been identified. Dracorhodin has a relatively greater abundance than 10 other compounds in E25–E100. Nevertheless, E0 contained more dracocephaline and dracorubin than the other nine compounds (Table 6). The E100 extract has the highest dracorhodin content compared with other extracts. The nordracorhodin compound was also highest in the E100 extract. Unlike the case with extract E100, extract E50 contains compounds (2S)-5,7-dihydroxy-dihydroflavone; dracorubin; and daemoflavan E larger than other extracts.

**Table 6.** The components of *D. acehensis* resin extracts.

| Compounds  | MW     | Relative Abundance (%) |       |       |        |        | Class of Compound |
|--|--------|------------------------|-------|-------|--------|--------|-------------------|
|  |        | E0                     | E25   | E50   | E75    | E100   |                   |
| Dracorhodin                                      | 266.09 | 0.011                  | 4.504 | 7.837 | 14.173 | 17.975 | Flavylium         |
| Nordracorhodin                                   | 252.08 | 0.001                  | 0.271 | 0.542 | 2.072  | 3.288  | Flavylium         |
| (2S)-5,7-Dihydroxy-dihydroflavone                | 256.07 | 0.000 *                | 0.841 | 1.675 | 1.496  | 1.510  | Flavonoid         |
| Dracorubin                                       | 488.16 | 0.377                  | 2.961 | 3.985 | 0.518  | 1.436  | Proanthocyanidin  |
| Daemoflavan E                                    | 286.12 | 0.000 *                | 1.745 | 2.535 | 1.825  | 1.323  | Flavonoid         |
| Daemoflavan G                                    | 282.09 | 0.000 *                | 0.281 | 0.634 | 0.663  | 0.822  | Flavonoid         |
| Dracoflavan B1                                   | 538.20 | 0.005                  | 0.018 | 0.050 | 0.207  | 0.204  | Biflavonoid       |
| (2R)-caesalflavan B                              | 286.12 | 0.000 *                | 0.001 | 0.002 | 0.245  | 0.203  | Flavonoid         |
| 4,6-Dihydroxy-2-methoxy-3-methylidihydrochalcone | 286.12 | 0.000 *                | 0.018 | 0.012 | 0.144  | 0.130  | Chalcone          |
| Daemoflavan H                                    | 268.07 | 0.000 *                | 0.013 | 0.058 | 0.098  | 0.129  | Flavonoid         |
| Dracocephaline                                   | 538.20 | 0.438                  | 0.002 | 0.003 | 0.002  | 0.004  | Biflavonoid       |

\* lower than 0.001.

#### 4. Discussion

The polarity and the type of solvent used significantly affect the yield of the extract obtained. In this study, the higher the ethanol composition in the ethanol–water mixture, the higher the extract yield. This indicates that the increasing polarity of the solvent (more water) does not increase the compound's solubility from the resin to the solvent. However, previous studies have shown that the yield produced from an extraction with nonpolar solvents (n-hexane) produces a very small yield, while the highest yield was obtained in an extraction with ethyl acetate [11]. Other studies also showed that a higher ethanol composition increased the extract yield of propolis, which was dominated by resin [17]. Based on these two phenomena, the components in the *D. acehensis* resin were compounds with medium polarity (semi-polar).

In line with the yield parameter phenomenon, the extract obtained with 100% ethanol (E100) as the solvent showed higher antioxidant capacities and SPF values than the other extracts. In comparison, the 100% ethanol extract of the *Gyrinops versteegii* leaf exhibited the highest antioxidant and SPF values [27], even though the value remained lower than what was obtained in this study. This indicates that important compounds that have antioxidant activity are very well dissolved in ethanol solvents. Flavonoid compounds are thought to be responsible for this antioxidant activity. Eight flavonoid compounds have previously been reported in the ethanol extract of *D. draco* resin and identified as single flavonoids and biflavonoids [7]. The flavonoid group of compounds has also been widely known to have good antioxidant activity [9]. A previous report also revealed that the flavonoid compound is a major constituent in extracts with good antioxidant activities [28].

The photoprotective activity of the extract based on the SPF value showed good potency of the E100 extract. Compared with the previous report using pomegranate juice [29], the *jernang* extracts and its phytosome showed higher SPF values. Using the classification from the FDA based on the SPF value, E100 was classified as a component with ultra-light protection (SPF value > 15). In line with the high antioxidant activity, the high SPF value was also thought to come from the flavonoid component in the resin extract of *D. acehensis*.

Phytosome-E100 with F1 formulation provided the highest antioxidant activity and SPF value, but the yield between formulations was not affected. The SPF values and antioxidant capacities of CUPRAC and ABTS of F1 phytosomes decreased compared with that of E100 but increased in the DPPH test. This decreased antioxidant activity was also observed in the phytosomes of persimmon extracts, but the phytosomes were able to maintain the antioxidant activity of the extracts compared with non-phytosome extracts with long-term storage [19]. Other studies have also shown that phytosomes increase the solubility, oral bioavailability, and pharmacological activity (especially antioxidants) of apigenin [30]. In addition, another study reported that phytosome formulations for propolis extract were able to increase their solubility and bioavailability and to maintain the antioxidant activity and SPF value of the extract [17].

In this study, the antioxidant parameter and SPF value were consistently correlated in either the extract or phytosome forms. Considering the high correlation coefficient among these parameters, we hypothesized that the constituent with good antioxidant activity has good properties for protection from sun radiation (UVA and UVB). Flavonoid and its derivative were thought to be the key constituents influencing this phenomenon, as this class of compounds is considered to have a high presence in resin extracts. Flavonoid has some action related to this phenomenon, namely antioxidant actions (ROS and RNS scavenging mechanisms), ultra-violet light absorption, and several signal pathway modulations [10].

Qualitative phytochemical analyses of E100 and F1 showed that the phytosomes retained the components of the compounds in the extract, which were characterized by the similarity of the qualitative composition of the components of the phytochemical compounds. The interaction between compounds formed in the phytosomes was also confirmed through the F1 FTIR spectrum. In line with the qualitative phytochemical results, the E100 spectrum with characteristic O–H, C=O, C–O (ether), and C=C aromatic

signals was a signal for compounds in resin extracts that are dominated by flavonoids and their derivatives [8,31,32]. Meanwhile, the soy lecithin spectrum showed the presence of C=O, P–O–C, and C–O signals characteristic of the signals of phospholipid compounds consisting of fatty acid unit (with ester groups) phosphates joined together [33]. The interaction between metabolite compounds and soy lecithin was thought to be a hydrogen interaction characterized by a broader O–H signal ( $3419.89\text{ cm}^{-1}$ ) in the spectrum of phytosome F1 [18,34].

A constituent analysis in the extract confirmed the class of compounds that presumed to be responsible for antioxidant activity, SPF value, qualitative phytochemical analysis, and the description of functional groups in FTIR. The composition of compounds based on LC-MS/MS analysis showed that the extract was contained by flavonoid compounds (including its derivatives and biflavonoids). Dracorhodin is a flavylum chromophore (a derivative of flavonoids), a characteristic compound of *Daemonorop* resins [6,35]. This compound was also thought to be one of the essential compounds for the high antioxidant capacity and SPF value of the E100 extract because of its higher amount than in other extracts. Other compounds that were not identified by LC-MS/MS but could be identified through qualitative analysis, such as alkaloids, phenyl hydroquinone, and terpenoids, were thought to have a significant influence on biological activities related to antioxidants and sun protection activities. Other compounds have also previously been identified in *Daemonorops* resins [7,36]. The dracorhodin, in the form of dracorhodin perchlorate, has antiproliferative activity and promotes wound healing [37,38]. However, no studies have specifically identified the photoprotective activity of the compounds in *D. acehensis* resin. Based on this research, the dracorhodin compound was also presumed to be an important compound in the antioxidant and photo protective activities of the ethanol extract and phytosome resin of *D. acehensis*.

## 5. Conclusions

The best extraction solvents and phytosome formulations were successfully obtained in this study. Extraction with an increasing ethanol solvent composition showed a linear trend with the yield, antioxidant activity, and SPF value. Purified ethanol was chosen as a promising solvent for *D. acehensis* resin extraction. The phytosome formulation of soy lecithin/E100 with a ratio of 1:1 exhibited good antioxidant activity and a high SPF value that could be maintained from the extract without phytosomes. Various phytochemical compounds were identified in the *D. acehensis* extract and its phytosome. In particular, the phenolic group (flavonoids and their derivatives). Dracorhodin was a compound that distinguishes *jernang* extracts. The increase in ethanol concentration also increased the percentage of dracorhodin in extracts thought to be one of the compounds responsible for their bioactivity. Therefore, this research provides new benefits for the broader development of *D. acehensis* resins in terms of health and cosmetics, especially using the best extraction solvents and their development in the form of phytosome.

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