

Article

Intsia bijuga Heartwood Extract and Its Phytosome as Tyrosinase Inhibitor, Antioxidant, and Sun Protector

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Abstract: *Intsia bijuga* (Colebr.) wood (Indonesian: Merbau) is commercial wood with high economic value and is most commonly found in Indonesia. *Intsia* wood extractives have biological activities related to their potential as natural active ingredients for antiaging cosmetics. This study aimed to select the best extraction solvent and phytosome formulation of *I. bijuga* heartwood extract as an active ingredient for topical antiaging cosmetics. There were five and three variations on extraction solvent and phytosome formulation, respectively. Three main antiaging activity parameters, namely antioxidant, antityrosinase, and sun protection factor (SPF) values, were considered in selecting the best extract and phytosome formula. The results showed that 50% ethanol possessed good antioxidant and antityrosinase activity, but was lower in SPF value, which was significantly different than in other extracts. The phytochemical profile revealed robidanol and robinetin as the main constituent in five *I. bijuga* extracts. Phytosome F3 possessed high antioxidant, antityrosinase, and SPF values compared to other 50% ethanol phytosome extracts. It could be concluded that *I. bijuga* ethanol extracts and its phytosome are potent enough to be developed as an antiaging active ingredient in topical use cosmetics.

Keywords: antiaging; extractives; heartwood; *Intsia*; robidanol; robinetin



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1. Introduction

Merbau wood (*Intsia spp.*) is a commercial wood with high economic value belonging to the Fabaceae family. Nine species of merbau are spread in several parts of the world. There are three species of merbau in Indonesia, namely *I. bijuga*, *Intsia palembanica*, and *Intsia acuminata*. *I. bijuga* (Colebr.) Kuntze is the most commonly found in Indonesia. This species is found from Sumatra to Papua, but around 84.4% of Indonesian merbau production comes from Papua [1]. This wood is mainly produced in Maluku and Papua, in quantities up to 1.98 million m³ [2]. Merbau wood is widely used as construction material, furniture, panels, carvings, truck bodies, musical instruments, etc.

Extractives from *Intsia* wood have biological activities related to their potential as natural active ingredients for antiaging cosmetics. Antiaging cosmetics must contain active ingredients that provide antioxidants and sun protection, maintain elasticity and skin rejuvenation, and are anti-inflammatory and skin-brightening (tyrosinase inhibition activity) [3]. The methanol extract of the heartwood of *I. palembanica* containing three isolate compounds (4-dehydroxyrobidanol, ampelopsin, and fustin) showed good anti-acne potential through inhibition of the lipase enzyme [4]. Robidanol and its derivative

(4'-dehydroxyrobidanol) from the ethyl acetate fraction of this wood extract inhibit the tyrosinase enzyme and melanin synthesis, meaning it can be used as a skin whitening agent [5]. In addition, *I. palembanica* extract has antioxidant activity [6]. Several studies have shown the extractive potential of *I. palembanica* wood, but there is little information regarding the bioactivity of *I. bijuga* extract. Previous studies showed that the bark of *I. bijuga* has antioxidant activity [7] and several flavonoid compounds. Its heartwood has robinetin as its main component [8].

The extractive components of *Instia* have been obtained by extraction/isolation methods in the past, such as by using methanol and ethyl acetate, which have been suspected to be less safe for health. To overcome this problem, the use and selection of eco-friendly solvents such as water and ethanol were developed in this study. Previous reports showed that a variation mixture of ethanol and water affected the yield, phenolic composition, and antioxidant activity [9]. It was therefore suitable to apply the variation of the ethanol-water mixture as an extraction solvent when selecting the best extraction solvent in this study.

In addition, this type of isolated flavonoid compound is a polar compound that is less soluble in fat (the bioavailability problem). The manufacture of phytosome complexes from soy lecithin and merbau extract was also developed to overcome the bioavailability problems. These phytosomes use a modification technology in the delivery system to bind extract components/bioactive compounds with phospholipid molecules. Several studies have shown that this modification effectively increases bioavailability for absorption in the body, increases surface penetration, and maintains bioactivity in the long term [10–12]. Therefore, this study aimed to select the best extraction solvent and phytosome formulation of *I. bijuga* heartwood extract for use as an active ingredient in topical antiaging cosmetics. As an antiaging agent, the important properties of the active compounds studied are antioxidant activity, sun protector factor (SPF), and tyrosinase inhibition activity.

2. Materials and Methods

2.1. Extraction

The sample of *I. bijuga* heartwood was harvested from Papua (Indonesia). These samples were obtained from three trees with diameters of 39, 44, and 49 cm that contained 78–86% heartwood. The tree species was confirmed by Herbarium Bogoriense, Lembaga Ilmu Pengetahuan Indonesia (LIPI) Biology Indonesia (letter number: B-47/IV/DI.01/I/2021). The heartwood was separated and powdered with 40–60 mesh (moisture content below 10%). Then, the powder was immersed in 100% ethanol (E100), 75% ethanol (E75), 50% ethanol (E50), 25% ethanol (E25), and water (E0) at room temperature. The solvent–powder ratio was 10:1. Extraction was successively carried out three times over 24 h each. The extraction process was carried out using three replications separately. The filtrate was separated and concentrated using a rotary evaporator (175 mbar at 40 °C for 2 h). Furthermore, the concentrated extract was weighed to obtain the extraction results and stored for further testing.

2.2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC was determined using Folin–Ciocâlteu assay [13]. Test solutions consisted of 10 µL of extract solution, 150 µL of aqua bidest, 10 µL of 10% Folin–Ciocâlteu reagent, and 20 µL of 10% Na₂CO₃ in a 96 well microplate. These test solutions were kept for 30 min at room temperature. The phenolic content was reported in milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract) by measuring absorbances of test solution and the positive control (gallic acid) at 750 nm using a microplate reader (Epoch Biotek, Winooski, VT, USA).

In TFC determination [13], a 60 µL of extract solution, 10 µL of 10% AlCl₃, 10 µL of CH₃COOK, and 120 µL of aqua bidest were mixed in a 96 well microplate. Then, the test solution was incubated at ambient temperature for 30 min. Measurements were carried out using a microplate reader at 415 nm. Quercetin was used as a positive control to create

a calibration curve and total flavonoid contents were reported as milligrams of quercetin equivalent per gram of extract (mg QE/g).

2.3. LC-MS/MS Analysis

Five merbau extracts were putatively identified for their components using UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS (Thermo Fisher Scientific, Waltham, MA, USA). Separation was performed in an Accucore C18 column, 100 × 2.1 mm, 1.5 µm (Thermo Fisher Scientific, Waltham, MA, USA) with a temperature of 30 °C. Gradient elution was used as a separation system, with 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). Eluents A and B were H₂O + 0.1% formic acid and acetonitrile + 0.1% formic acid, respectively. The flow rate was maintained at 0.2 mL/min. Molecular fragmentation (mass spectrometry) was carried out using electrospray ionization with an ionizer (3.80 kV, capillary temperature 320 °C, negative ionization mode) with a resolution of 70000. Mass fragment readings were carried out with a scan range of 100–1500 m/z. The data acquisition was carried out using Xcalibur 4.2 software (Thermo Fisher Scientific, Waltham, MA, US). Identification of compounds was carried out by comparing the mass fragmentation of the compounds detected in the sample with the database from the Mass Spectrometry Data Center (National Institute of Standards and Technology, Waltham, MA, USA) built in Xcalibur software.

2.4. Antioxidant Activity

The antioxidant activity of extracts using the DPPH scavenging activity assay refers to the procedure of [14]. Measurements were made by mixing 100 µL and 100 µL of DPPH solution (4 mg DPPH in 100 mL ethanol) into a 96 well microplate. The test solution was then incubated for 30 min at room temperature in a dark room. Next, absorbance was measured at a wavelength of 517 nm using a microplate reader (Epoch Biotek, Winooski, VT, USA). Trolox was used as a positive control/comparison. The value of antioxidant activity was reported as a concentration value at 50% radical inhibition (IC₅₀).

2.5. Tyrosinase Inhibition Activity

We followed the procedure described by Nerya et al. (2003) [15] with modifications for determination of tyrosinase inhibition activity. Eighty µL of phosphate buffer solution (50 mM; pH 6.5), 40 µL of substrate (L-tyrosinase) solution, 40 µL of tyrosinase enzyme solution, and 40 µL of sample solution were put into a 96 well microplate. The reaction used was the monophenolase reaction. The mixture was homogenized and incubated for the optimum incubation time at 25–30 °C. The absorbance of each test solution was calculated with a microplate reader (Epoch Biotek, Winooski, VT, USA) at the maximum wavelength. Kojic acid was used as a positive control because it is a tyrosinase inhibitor with the highest inhibition and stability in skin-lightening cosmetics [16]. The inhibitory activity was reported in the form of IC₅₀.

2.6. SPF Value Determination

Determination of SPF value used a Ultra Violet-Visible (UV-Vis) spectrophotometer at a wavelength of 290–360 nm using a test solution with a concentration of 125 mg/L and ethanol as a blank. Absorption data were read at 2.5 nm intervals. The measurement was repeated three times. SPF values were counted using equation [10]:

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

Cf: 10 (constant), *EE*: erythemogenic effect, *I*: intensity of the photon, *Abs*: absorbance of the samples.

2.7. Phytosome-Extract Complexation

Phytosomes were manufactured referring to the research of Singh and Narke (2015) [17]. Three formulations of phytosomes were made with the weight/weight ratios of extract/soy lecithin, namely F1 (1:1), F2 (1:2), and F3 (2:1). The mixture of solid extract and soy lecithin was 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 20 mL of n-hexane was added with continuous stirring to obtain a precipitate. The precipitate was separated and stored in a vacuum desiccator for 12 h. The precipitate was further pulverized and filtered through a 100-mesh sieve. The powder was stored in a dark glass vial before being used for bioactivity measurement and characterization.

2.8. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The most active extract and phytosome, as well as soy lecithin, were analyzed for their FTIR spectra to detect and determine the chemical bonds/functional groups detected in the extract and phytosomes of the *I. bijuga* extract. The Bruker Tensor 37 FTIR spectrophotometer equipped with deuterated triglycine-sulphate (DTGS) as detector (Bruker, Karlsruhe Germany) using the KBr-pellet system was used to analyze the spectra. The absorbances were measured in the wavenumber range of 600–4000 cm^{−1} with a resolution of 4 cm^{−1} and 32 scans.

2.9. Statistical Analysis

The yield, TFC, TPC, antioxidant, antityrosinase, and SPF values were analyzed using an analysis of variance with a completely randomized trial design using SPSS 25. Duncan's multiple range test was also used to identify the significance value among groups. The analysis aimed to examine the influence of the types of solvent and phytosome formulations on the yield, TFC, TPC, antioxidant, antityrosinase, and SPF value. The levels of treatment differed in the concentration of ethanol as the extraction solvent, namely 100% ethanol (E100), 75% ethanol (E75), 50% ethanol (E50), 25% ethanol (E25), and distilled water (E0), as well as phytosome formulation (F1, F2, and F3). In addition, Pearson's correlation coefficient was also determined to evaluate the correlation among parameters.

3. Results

3.1. Yield, Bioactivity, and Phytochemical Profile of Extracts

The analysis of variance showed that the type of extraction solvent affected the yield, TPC, and TFC of *I. bijuga* extracts (Table 1). The E50 extract had the highest percentage of yield compared to other extracts. However, this trend was different for TPC and TFC. The E100 extract contained phenolic compounds that were larger than other extracts, while the E0 extract contained many flavonoids.

Table 1. Yield, phenolic, and flavonoid content of *I. bijuga* extracts.

Extracts	Yield (%)	TPC (GAE/g Extract)	TFC (QE/g Extract)
E100	12.81 ± 1.21 d	1238.96 ± 3.61 a	1.63 ± 0.00 c
E75	16.13 ± 0.20 b	859.80 ± 7.22 b	2.53 ± 0.00 b
E50	17.79 ± 0.48 a	768.13 ± 10.83 c	1.58 ± 0.004 d
E25	14.69 ± 0.90 c	570.21 ± 3.61 d	0.71 ± 0.00 e
E0	11.11 ± 0.36 e	491.04 ± 7.22 e	3.12 ± 0.02 a

Numbers followed by the same letter represent values that are not significantly different ($p > 0.05$).

Variation of the ethanol concentration significantly influenced the antioxidant, antityrosinase, and SPF value (Table 2). The E50 extract had the highest antioxidant and antityrosinase activity, and was significantly different from other extracts. In comparison with trolox as a positive control, all extracts had better activity. The same phenomenon also occurred in the inhibition of the tyrosinase enzyme with kojic acid as a positive control. The SPF value showed a different trend compared to the antioxidant and antityrosinase

activities. The increase in the ethanol concentration was in line with the increase in the SPF value.

Table 2. Antioxidant, tyrosinase inhibition, and SPF value of *I. bijuga* extracts.

Extracts	IC ₅₀ (mg/L)		SPF Value
	Antioxidant	Antityrosinase	
E100	9.37 ± 0.11 b	11.61 ± 0.17 b	16.84 ± 0.01 a
E75	8.17 ± 0.16 c	11.17 ± 0.29 bc	13.25 ± 0.13 b
E50	5.68 ± 0.48 d	10.36 ± 0.16 d	12.36 ± 0.99 c
E25	5.72 ± 0.11 d	10.97 ± 0.27 c	8.33 ± 0.1 d
E0	6.07 ± 0.13 d	11.23 ± 0.33 bc	6.22 ± 0.04 e
Positive control	* 9.78 ± 0.08 a	** 31.33 ± 0.25 a	-

Numbers followed by the same letter represent values that are not significantly different ($p > 0.05$). * trolox; ** kojic acid.

The best extract was selected by considering the antioxidant activity, antityrosinase activity, SPF value, and the yield of extraction. The E50 was considered the best extract to be formulated as a phytosome. This extract was chosen for its good antioxidant and antityrosinase activity, although the SPF value was lower. In addition, this extract also had a higher yield, so in its utilization for phytosomes it can provide more raw materials.

Using different ethanol concentrations as the extraction solvent resulted in variations in the composition of the detected compound. LC–MS/MS analysis showed that (–)-robidanol was the dominant compound in all extracts (Table 3). The increase in ethanol concentration did not result in an increase in the abundance of (–)-robidanol. The abundance trend of (–)-robidanol from low to high was E75 < E50 < E100 < E25 < E0. Robinetin and catechin were two predominant compounds in the extract. Robinetin became the compound with the second-largest abundance in the E25–E100 extract, while the E0 extract contained many catechins with a small amount of robinetin. The results of this identification also showed that the extract of *I. bijuga* contained high levels of flavonoids.

Table 3. Chemical constituents of *I. bijuga* extracts.

Compound Name	Retention Time (min)	Compound Class	Relative Abundance (%)				
			E0	E25	E50	E75	E100
(–)-Robidanol	4.787	Flavonoid	57.8	47.8	46.3	40.7	47.4
Robinetin	6.250	Flavonoid	2.8	28.6	31.5	35.0	29.5
Catechin	1.953	Flavonoid	20.8	3.9	4.6	4.0	4.8
Naringenin	10.784	Flavonoid	0.6	3.2	3.0	3.5	3.3
Dihydromyricetin	5.499	Flavonoid	1.4	4.6	2.3	2.3	1.9
Piceatannol	6.737	Stilbene	0.1	2.9	1.8	2.4	2.4
Quercetin	9.127	Flavonoid	0.1	1.0	1.9	2.1	1.4
Fustin	7.470	Flavonoid	0.8	1.3	1.4	1.3	1.8

Based on the data for each compound in all extracts, the five extracts actually had similar chemical profiles and there were only differences in their content. This can also be observed in the five chromatogram patterns of the *I. bijuga* extracts (Figure 1). The similarity of the peak pattern on the chromatogram describes the same time retention, which represents the same compound. The differences previously presented in Table 3 were observed by the presence of differences in peak height. This peak height described the relative abundance associated with the amount of the compound in the sample.

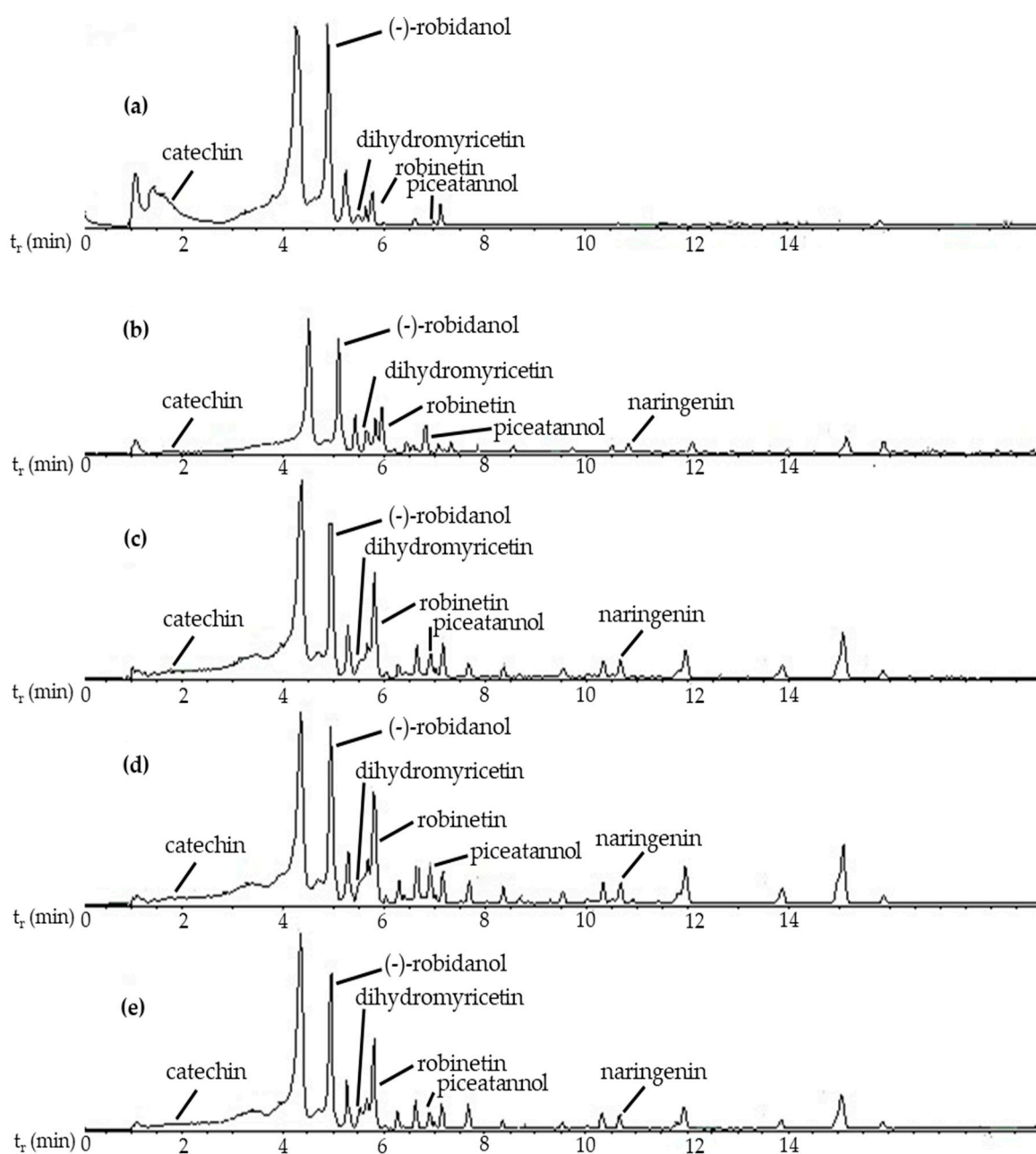


Figure 1. Chromatogram profile of five *I. bijuga* extracts. (a) E0, (b) E25, (c) E50, (d) E75, and (e) E100.

3.2. Yield, Bioactivity, and Phytochemical Profile of Phytosome

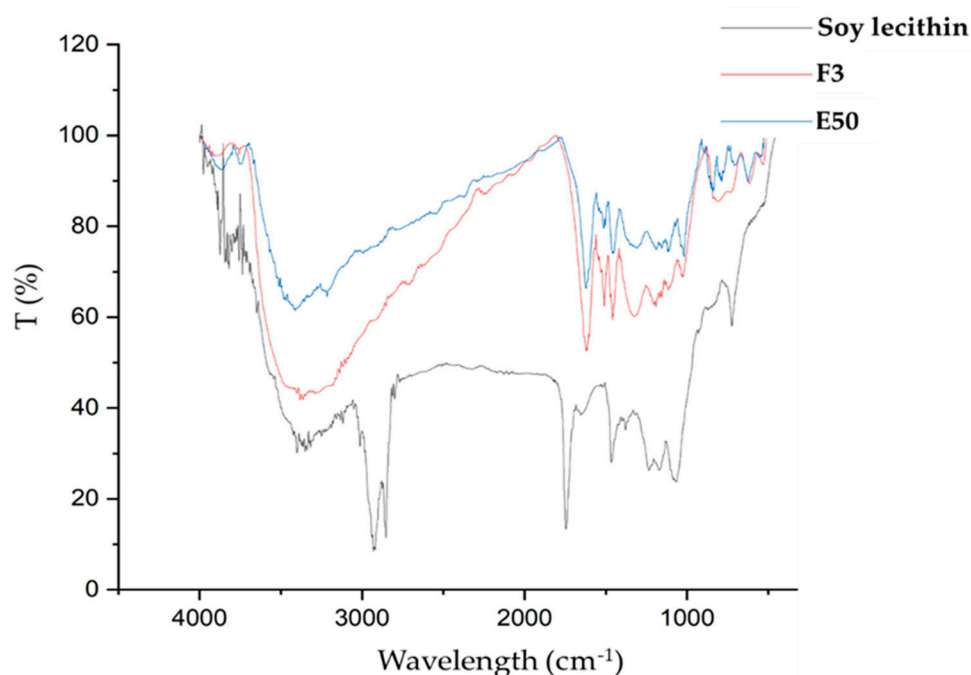
Different formulations of phytosomes resulted in phytosome-E50 complexes with different antioxidant, antityrosinase, and SPF values. The antioxidant and antityrosinase activities were lower than the extract without phytosome. However, extracts with phytosome had higher SPF values (Table 4). Phytosome F2 showed a higher yield value, but this was in contrast to its biological activity. Antioxidant activity, tyrosinase enzyme inhibition, and the SPF value of F2 were lower than F1 and F3. Among the three phytosome formulas, F3 was the best formula with good antioxidant activity, tyrosinase inhibition, and good SPF values. Although the biological activity of F3 was not significantly different from that of F1, the F3 formula produced a higher yield.

Table 4. Yield, antioxidant, antityrosinase, and SPF value of phytosome-E50.

Extracts	Yield (%)	IC ₅₀ (mg/L)		SPF Value
		Antioxidant	Antityrosinase	
F1	69.87 ± 1.69 b	15.27 ± 0.34 b	33.40 ± 0.05 b	28.13 ± 0.32 a
F2	83.20 ± 1.17 a	16.19 ± 0.35 a	74.66 ± 1.08 a	24.91 ± 0.11 b
F3	76.02 ± 4.92 b	15.03 ± 0.30 b	33.36 ± 0.33 b	27.78 ± 0.16 a
Positive control		* 9.78 ± 0.08	** 25.56 ± 0.57	

Numbers followed by the same letter represent values that are not significantly different ($p > 0.05$); * trolox; ** kojic acid.

Qualitative analysis through the FTIR spectrum showed a combination of E50 spectrum and soy lecithin in phytosome (Figure 2). E50 extract based on LC-MS/MS analysis was composed predominantly of flavonoid compounds and a small number of stilbenes. The main characteristics of the structure of flavonoids are hydroxyl functional groups ($3200\text{--}3800\text{ cm}^{-1}$), carbonyl (1622 cm^{-1}), tertiary hydroxyl/phenol ($1190\text{--}1458\text{ cm}^{-1}$), ether (1117 cm^{-1}), and aromatic groups ($623\text{--}841\text{ cm}^{-1}$). The characteristic absorption bands of soy lecithin were found at $2852\text{--}2930\text{ cm}^{-1}$ (fatty acid alkane chain), $1653\text{--}1745\text{ cm}^{-1}$ (C=O ester), 1322 cm^{-1} (P=O phosphate), 1172 cm^{-1} (P-O-C), and 1023 cm^{-1} (N-CH₃). These bands were observed again in the F3 spectrum, namely hydroxyl ($3928, 3381\text{ cm}^{-1}$), carbonyl (1614 cm^{-1}), P=O (1322 cm^{-1}), OH-phenol (1190 cm^{-1}), POC & ether (1115 cm^{-1}), N-CH₃ (1023 cm^{-1}), and aromatic carbon ($532\text{--}808\text{ cm}^{-1}$).

**Figure 2.** FTIR spectrum of E50, soy lecithin, and F3.

3.3. The Correlation among Parameters

Pearson's correlation coefficient was used in this study to correlate the data of each parameter and identify the correlation of each parameter. Based on Figure 3, three bioactivity parameters, namely DPPH radical cleavage, tyrosinase (Tyro) enzyme inhibition, and SPF values, had a significant correlation. The DPPH and Tyro parameters had a positive and significant correlation, which indicates that the data trends between the two parameters were similar. The SPF value had a significant negative correlation with the DPPH and Tyro parameters. This indicated that the trend of the SPF value data has an inverse similarity; a high SPF value will be accompanied by a low IC₅₀ value.

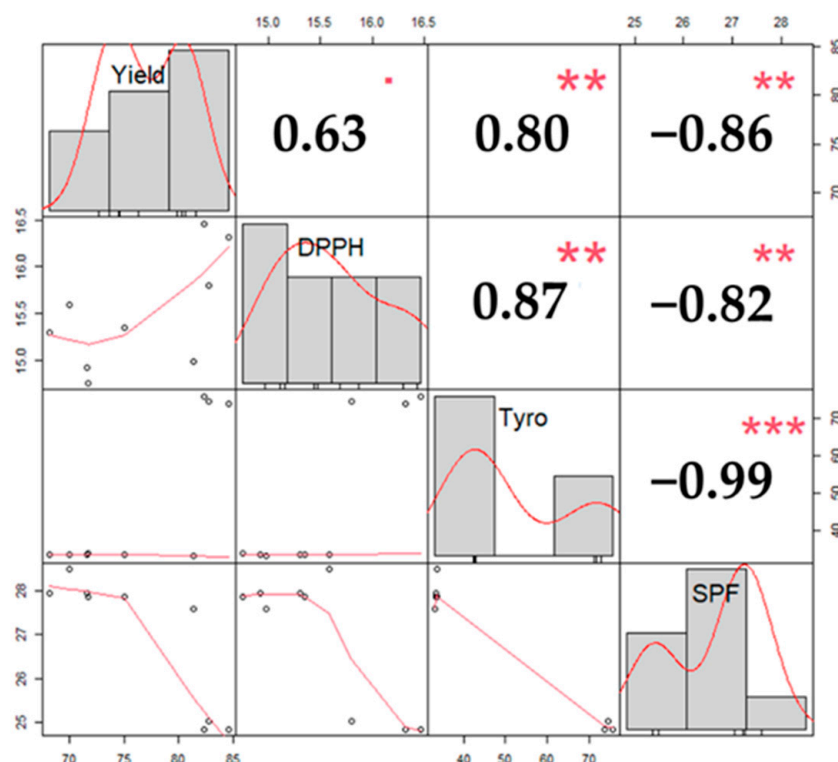


Figure 3. Pearson's correlation matrix. The diagonal, top right, and bottom left boxes represent the data distribution of each variable, correlation coefficient, and bivariate scatter plot, respectively; *** $p < 0.001$, ** $p < 0.01$.

The abundance of compounds in each extract can also be related to the parameters of radical scavenging activity, tyrosinase inhibition, and SPF values. Based on Table 5, the characterized compounds (robidanol and robinetin) did not significantly correlate with the parameters of DPPH, Tyrosinase inhibition, and SPF value. A significant correlation was only found in the compound of quercetin and DPPH parameters but this compound's level was very low. When comparing the correlation between robinetin and robidanol data, the increase in robinetin abundance correlated with increased antioxidant activity and inversely with robidanol. A different phenomenon occurred in the SPF parameter: the increase in robidanol correlated with the increase in the SPF value. In addition, the TPC parameter showed a high and significant positive correlation with DPPH and SPF (Table 5).

Table 5. Pearson's correlation coefficient of identified compounds, TPC, and TFC to the bioactivity parameters of *I. bijuga* extracts.

Compound/Parameter	DPPH	Tyrosinase Inhibition	SPF
(-)-Robidanol	0.85	0.63	0.74
Robinetin	−0.85	0.7	−0.75
Catechin	0.77	0.65	0.71
Naringenin	−0.79	−0.57	−0.79
Dihydromyricetin	0.11	0.14	0.00
Piceatannol	−0.54	−0.38	−0.66
Quercetin	−0.96 *	−0.79	−0.73
Fustin	−0.77	−0.45	−0.87
TPC	0.87 ***	0.40	0.97 ***
TFC	0.14	0.24	−0.21

*** $p < 0.001$, * $p < 0.05$.

4. Discussion

The combination of water and ethanol as a solvent in this study did not show an increasing yield trend as the ethanol concentration increased. The compounds in *I. bijuga* were thought to be soluble in ethanol and water in equal amounts. This indicated that *I. bijuga* consists of varying polarities because the highest yield was obtained from extraction with an ethanol–water ratio of 1:1. Previous reports show that 50% ethanol also produces higher yields than 100% and 75% ethanol [18,19]. The content of phenolic compounds corresponding to the increase in ethanol in the extraction solvent has also been observed previously [18]. In contrast, flavonoids were contained more in aqueous extract. Previous research using several types of plants also confirmed this phenomenon [20].

All types of extracts of *I. bijuga* had good radical scavenging and tyrosinase inhibitory activity, as indicated by their activity exceeding the positive control in each case. When comparing the content of compounds in extract, the flavonoid component in the extract played an essential role. All extracts were dominated with robidanol, which provided good tyrosinase inhibitory and radical scavenging activity. Robidanol, epirobidanol, and dehydroxyrobidanol have been isolated from *I. palembanica* wood and have better tyrosinase inhibition than kojic acid [5]. The information regarding the antioxidant activity of *I. bijuga* extract is still limited. From the IC₅₀ values, all types of extracts had very strong antioxidant activity. Flavonoids were the main components of this extract and were considered to play an essential role in the antioxidant activity of the extract. [21].

The SPF values were influenced by the increase in the ethanol concentration in the extraction. The effect of increasing the concentration was also related to the TPC value. The increase in levels of phenolic compounds was thought to increase light protection (SPF). Previous studies have shown that the levels of phenolic compounds are relatively correlated with the SPF value [22], but the values obtained were not as high as those obtained in this study.

Modification with phytosome significantly affected the biological activity among the formulations tested and the change in activity compared to non-phytosome extracts. In this study, a decrease in antioxidant activity and tyrosinase inhibition were observed. However, there was an increase in the SPF value. The decrease in the activity was also observed in previous studies [11], though other studies have shown that modification with phytosomes provides prevention of degradation and maintains the biological activity of extracts in long-term storage [12]. In addition, in vivo testing and clinical trials reveal that phytosome modification can increase bioavailability so that the bioactive components can be absorbed and the effect of these bioactive components is more visible. Phytosome-apigenin tested on CCl₄-treated rats shows a better antioxidant effect than apigenin without phytosome, which is observed by its effect decreasing several enzymes associated with antioxidation in rat liver [23]. In addition, a clinical trial shows bergamot phytosome decreases visceral adipose tissue, total cholesterol, and LDL cholesterol levels with supplementation for 30 days [24]. Furthermore, phytosome modification in *I. bijuga* extract may positively affect topical use, and further in vivo testing or clinical trials need to be carried out to confirm this.

In this study, a formulation of phytosome with *I. bijuga* extract was successfully carried out. Soy lecithin and compounds in the most active extract (E50) interacted through non-covalent interactions (hydrogen bond). This could be confirmed through the widening of the hydroxyl absorption at a wavelength of 3500 cm^{−1} [25].

The correlated data among parameters either in extract or phytosome form revealed the statistical connection between chemical profile and bioactivity. Considering the high correlation coefficient among three main parameters (DPPH, tyrosinase inhibition, and SPF value), phenolic compounds (particularly flavonoid) were responsible for good antioxidant activity, inhibiting tyrosinase, and protecting from sun radiation. This class of compound was considered due to its high content in extracts. Previous work reveals that flavonoids possess some action related to this phenomenon, namely antioxidant action (ROS and RNS scavenging mechanism), absorbing ultra-violet lights, and modulating several signal pathways [26]. Another report showed a good correlation among antioxidant parameters

with TPC and TFC [27]. In addition, plants with potent antityrosinase activity also show a good correlation between antioxidant activity and TPC.

Robidanol and robinetin were key compounds in *I. bijuga* extracts. Regarding our Pearson's coefficient correlation results, it might be concluded that these compounds have contrary actions. Robidanol was presumed to be the main constituent for sun-protecting activity, whereas robinetin was considered the main constituent for scavenging radical activity. In terms of their molecular structure, these compounds have differences in the C-ring (Figure 4). Robidanol is a flavan-3-ols, while robinetin is a type of flavonol. The presence of ketones or double bonds in the C-ring structure is thought to have a major influence on the compound's activity. This different role has also been reported in a previous study, where these compounds exhibited antityrosinase activity with different effects, with robidanol having a very strong inhibitory effect, while robinetin had a very weak inhibitory effect [5].

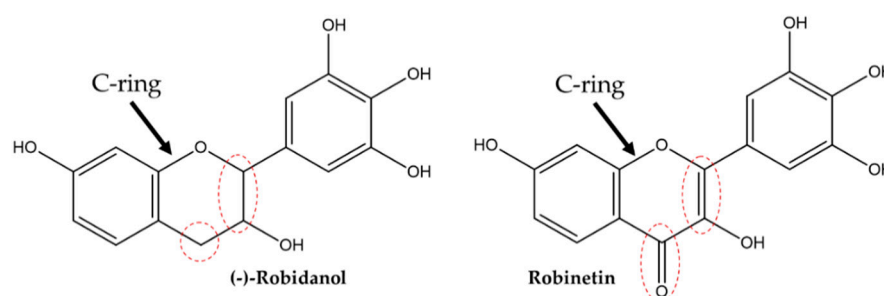


Figure 4. The difference between the molecular structure of robidanol and robinetin.

5. Conclusions

Variations in the extraction solvent and phytosome formulas significantly affected the phytochemical profile and antiaging activity parameters (antioxidant, antityrosinase, and SPF value) of *I. bijuga* extracts and its phytosome. The 50% ethanol extract and its phytosome (F3, extract: soy lecithin with the ratio of 2:1) were selected as the best extract and phytosome formula, respectively. Apart from their bioactivity, these extracts and phytosome were chosen because of their high yields. The accomplishment of making F3 phytosomes has been confirmed by the FTIR spectrum, which showed an interaction between the extract constituents and soy lecithin. The relationship between phytochemical profiles and antiaging activity parameters shows the relationship between robidanol and robinetin compounds as dominant components, both of which are thought to have different mechanisms of action. This finding provides information regarding the potential of forest products, especially wood extractives, in the health and cosmetic fields.

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