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Antioxidant Activity of Hydrophilic Cream of Methanol Extract of White Champaca Flowers (*Magnolia alba*)

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Artikel Penelitian

Abstract: *The white champaca flower (Magnolia alba) holds cultural significance for the Balinese society, being employed in religious ceremonies, adorning bridal setting, and enriching the aroma of incense. Moreover, white champaca flowers contain alkaloids, steroids, terpenoids, flavonoids, and phenols that have antioxidant activity. Therefore, it is interesting to determine the antioxidant activity of methanol extract of white champaca flowers and formulated it into an oil in water cream in this study. The extract was formulated into three cream formulas, FI, FII, and FIII with concentrations of 4, 8, and 12%, respectively. Antioxidant activity was assessed using DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay with UV-Vis spectrophotometer at a wavelength of 516 nm. The antioxidant activity based on IC_{50} values for FI, FII, and FIII were 29.72, 25.50, and 25.05 µg/ml, respectively, with IC_{50} value of vitamin C was 2.93 µg/ml as a standard comparison. The research findings indicate that the methanol extract of white champaca flower o/w cream exhibit a very strong antioxidant activity in vitro. Further research is imperative to investigate the ability of the formulations to delaying the photoaging mechanism through in vivo studies.*

Keywords: *antioxidant, cream, DPPH, IC_{50}, Magnolia alba*

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Abstrak: Bunga cempaka putih (*Magnolia alba*) memiliki makna budaya bagi masyarakat Bali, yaitu digunakan dalam upacara keagamaan, hiasan pengantin, dan memperkaya aroma dupa. Selain kegunaan tersebut, bunga cempaka putih mengandung alkaloid, steroid, terpenoid, flavonoid, dan fenol yang beraktivitas antioksidan. Dengan demikian sangat menarik untuk menentukan aktivitas antioksidan dari ekstrak metanol bunga cempaka putih yang diformulasikan ke dalam krim minyak dalam air (m/a) dalam penelitian ini.. Ekstrak metanol bunga cempaka putih diformulasikan ke dalam tiga formula krim, FI, FII, dan FIII, dengan konsentrasi masing-masing 4, 8, dan 12%. Aktivitas antioksidan dilakukan dengan metode DPPH (2,2-Difenil-1-Pikrilhidrazil) dengan menggunakan spektrofotometer UV-Vis pada panjang gelombang 516 nm. Aktivitas antioksidan berdasarkan nilai IC_{50} untuk FI, FII, dan FIII adalah 29,72 µg/ml, 25,50 µg/ml, dan 25,05µg/ml, sedangkan nilai IC_{50} antioksidan pembanding, vitamin C, adalah 2,93 µg/ml. Hasil penelitian menunjukkan bahwa krim m/a ekstrak metanol bunga cempaka putih memiliki aktivitas antioksidan yang sangat kuat secara *in vitro*. Penelitian lebih lanjut diperlukan untuk mengeksplorasi kemampuan formula ini dalam memperlambat mekanisme penuaan akibat paparan cahaya matahari melalui studi *in vivo*.

Kata kunci: antioksidan, DPPH, krim, IC_{50}, *Magnolia alba*

Introduction

Nowadays, green product in cosmetic has become a trend. Plant extracts are widely recognized for their numerous beneficial activities, including antibacterial, antifungal, antiinflammation, antioxidant, among others. These activities were attributed by the presence of secondary metabolites such as alkaloid, flavonoid, tannin, steroid, and terpenoid. Among the recent developments in green products, antioxidant creams have gained significant attention. Antioxidant are highly sought-after for skin or body care due to the detrimental effects of UV light and skin aging issue. UV light exposure leads reactive oxygen species (ROS), disrupts the cellular homeostasis, and upregulate the expression of matrix metalloproteinase (MMP), resulting in decreased collagen synthesis and various skin problem such as wrinkles, dryness, hyperpigmentation, skin roughness, and photoaging (1,2). In order to counteract photoaging process, photoprotective compounds such as polyphenols and flavonoid are employed. Champaca flowers, both white and yellow, are well-known as antioxidant which can scavenge free radical, due its alkaloid, steroid, terpenoid, flavonoid, and phenol (3)

Champaca flower, an evergreen tropical plant thriving in Indonesia, possess a distinct fragrance that rapidly permeates the surroundings and commonly harvested in the evening and at dawn. The local residents utilize these flowers as fragrance, aromatherapy, essential oil, and ritual ceremony ingredient. Moreover, extensive studies have revealed various beneficial activities associated with white champaca flowers (*Magnolia* alba), including antibacterial, antifungal, antidiabetic, anti-inflammatory, antioxidant, tyrosinase inhibition, and photoprotective activities (3–7). *Magnolia alba* contain (-)-N-formylanonaine which exhibits DPPH free radical scavenging activity with an IC_{50} value of 121.4 µM (8). Furthermore, extracts of *M.alba* have demonstrated the ability to attenuate the expression of MMP induced by UV-B exposure, thereby restoring total collagen synthesis (9).

Despite limited reports on the application of these extracts in topical preparations, the available data has motivated us to develop an o/w cream formulation utilizing the methanol extract of *Magnolia alba*. The choice of this cream type is based on the hydrophilic characteristics, which enhances the penetration of active substance to the skin tissue and leading to optimal effects. In topical preparations, the active substance must be released from the base and diffuse to the surface of skin tissue. However, a more viscous base can hinder the diffusion process and impede optimal performance (10).

In this research, we determined the antioxidant activity of hydrophilic cream containing the methanol extract of white champaca flower (*Magnolia alba*) using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which evaluated by IC_{50} value.

Materials and Methods

Materials and Equipment

The equipment utilized were standard laboratory glassware (PT. Iwaki Glass Indonesia, Sumedang, Indonesia), Ohaus analytical weight scale (Shanghai, China), Philips mixer (Netherland), Buchi R 300 rotary evaporator (Flawil, Switzerland), Shimadzu UV-1800 UV-Vis spectrophotometer (Tokyo, Japan), and other supporting tools.

The materials were fresh white Champaca flower collected on 5 am at Semarapura district, Klungkung, Bali. Other materials utilized in this study included cetyl alcohol (Merck, USA), adeps lanae (Merck, USA), liquid paraffin (Brathacem, Indonesia), stearic acid (Bratachem, Indonesia), nipasol, glycerin, triethanolamine (SABA, Indonesia), aquadest, methanol (SABA Indonesia), DPPH (Sigma Aldrich, Indonesia), and vitamin C (Sigma Aldrich, Indonesia).

Methods

Plant Determination and Preparation of Extract

Plant was identified in Karakterisasi Kebun Raya "Eka Karya" Bedugul, Bali-BRIN. The sample was shade dried and grinded in mixer. The powdered flowers (696 g) were extracted by maceration with methanol (1 : 10) for three days

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with regular shaking. The extract was filtered and evaporated with rotary evaporator at 50° C to obtained methanol extract of white Champaca flowers. The crude extract was stored at -20° C until further use.

Cream Formulation

Cream was formulated in o/w or hydrophilic cream type. Cream was prepared in three formulas with varying concentrations of the methanol extract of white champaca flower FI (4%), FII (8%), and FIII (12%). The cream base was divided into two main phases, namely the oil and the water phase. The oil phase consisted of cetyl alcohol (2%), adeps lanae (2%), liquid paraffin (1%), stearic acid (5%) and nipasol (0.5%). The water phase consisted of nipagin (0.5%), glycerin (6%), triethanolamine (TEA) (1%), and aquadest ad 100 ml. Both phases were separately placed in porcelain containers and heated on a water bath until reaching 70° C. Subsequently, the oil phase was added to the water phase and stirred continuously in mortar to form cream base. Meanwhile, in a separated mortar, the methanol extract of white champaca flowers was prepared with adding the remaining unheated TEA and glycerin. The mixture was stirred continuously until no longer adhered to the mortar. The cream base then added gradually into the extract and stirred until homogenous. The final hydrophilic cream with methanol extract of white champaca flower placed in container and keep at room temperature.

Antioxidant study

The antioxidant activity of cream was assessed by stable DPPH free radical. DPPH is a stable free radical in room temperature by delocalization of the spare electron over the molecule. The delocalization was characterized by deep violet color with the maximum wavelength between 515-520 nm (11). The DPPH will accept a hydrogen atom from the antioxidant-containing sample, resulting the conversion of reactive 2,2-diphenyl-1-picrylhydrazyl into non-reactive 2,2-diphenyl-1-picrylhydrazyne and subsequent decolorization (pale yellow) corresponding to the number of captured electrons. Each sample was diluted with methanol into five variation concentrations (10, 20, 30, 40, and 50 ppm). Ascorbic acid as a standard of antioxidant activity was prepared in five different concentrations (2, 3, 4, 5, and 6 ppm) by pipetting 1 ml; 1,5 ml; 2 ml; 2,5 ml; and 3 ml of ascorbic acid solution 100 ppm. 2 ml of each concentration in each cream sample was combined with 2 ml of DPPH solution 40 ppm, followed by incubation for 30 minutes in a dark room at room temperature. The same procedure was applied to methanol as a control, and ascorbic acid solution as standard. The absorbance of solution was measured using UV-Vis spectrophotometer at 516 nm. The scavenging activity was calculated using the following equation:

$$\text{Scavenging activity}(\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\ x\ 100\%$$

The antioxidant activity was assessed by IC_{50} (Inhibition concentration) value, in which the smaller of IC_{50} value indicates the stronger of antioxidant activity. The IC_{50} was determined by substituted the y equation from linear regression between scavenging activity and concentration of solution on each sample and standard with 50 (12).

Results and Discussion

Antioxidants scavenge free radical by donate a hydrogen atom to reactive DPPH so it converts into non-reactive diphenyl picrylhydrazine, indicated by decolorization into pale yellow. The antioxidant activity of cream was listed in figure 1 and 2. Scavenging activity of sample in a concentration dependent manner (13). The higher concentration of extract in cream, the higher of the scavenging activity, which indicates that more antioxidant contained in the preparations are able to reduce the free radical (14,15).

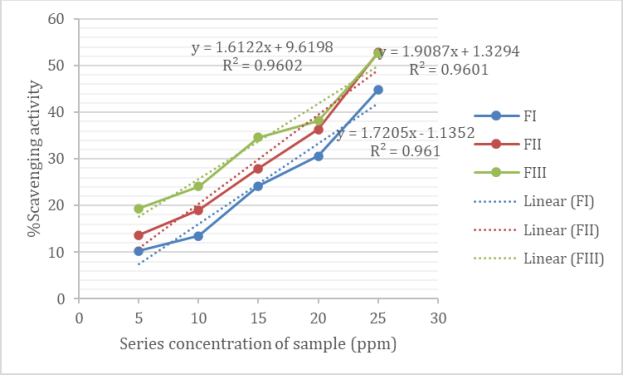

Figure 1. Scavenging Activity of Hydrophilic Cream of methanol extract of white champaca flower in 2-2-Diphenyl-1-Picrylhydrazyl Assay.

The antioxidant activity of each sample and standard was evaluated by linear regression analysis using five series of concentrations. The scavenging activity was calculated based on the aforementioned equation. The assessment of antioxidant activity was determined by the IC_{50} value, wherein a smaller IC_{50} value indicates a higher antioxidant activity in terms of hydrogen donation and free radical scavenging (16). Antioxidants with IC_{50} values below 50 ppm are classified as very strong, 50-100 ppm as strong antioxidant, 150-200 ppm as weak antioxidant, and > 200 ppm indicates as very weak antioxidant (17).

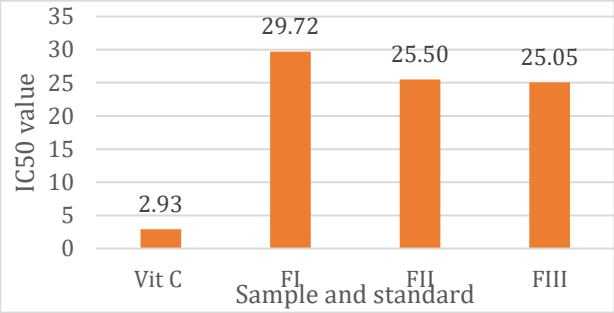

Figure 2. Comparative Graph of Inhibition Concentration Values of Hydrophilic Cream of methanol extract of white champaca flower.

Based on Figure 2, the IC_{50} value of FI, FII, and FIII were 29.72, 25.50, and 25.05 µg/ml, respectively, compared with ascorbic acid as a standard with IC_{50} value of 2.93 µg/ml. The sample were classified has a very strong antioxidant. Methanol was selected as the solvent for maceration due to its high dielectric constant, good polarity and solubility for phenolic compound (18). Previous studies proved that medicinal plant contain phytochemicals that responsible for the antioxidant activity. *Magnolia alba* contained carbohydrates, alkaloid, terpenoids, flavonoids, tannins, steroids, and phenol (7). Flavonoids, as a type of phenolic compound, possess redox properties that enable them to absorb and neutralize free radical, quench singlet and triplet oxygen, chelate the metal ions, and decompose peroxides (18). The phenolic compounds have the ability to delay aging process and exhibit photoprotection by inhibiting oxidative stress and inflammation induced by UV irradiation (19). In previous study about edible flowers in China, *M.alba* flowers was extracted using the mixture of acetone/water/acetic acid (70:29.5:0.5). The antioxidant activity of the sample was reported 58.22 ± 5.57 µmol Trolox Equivalent (TE)/g in DPPH method on a dry weight basis; 111.54 ± 7.47 µmol TE/g in 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate (ABTS) method; and 15.51 ± 1.05 mmol of Fe^{2+}E/100 g samples in ferric reducing antioxidant power (FRAP) method. The total phenolic compound (TPC) of the extract 18.9 ± 10.6 mg GAE/g with total flavonoid compound (TFC) 5.56 ± 0.33 mg CAE/g. The TPC exhibited a positive correlation with antioxidant capacity of edible flowers (20). *Magnolia alba* reported contained metabolites such as (-)-N-formylanonaine, α-terpineol, linalool, and geraniol. The antioxidant activity of α-terpineol was found to have an IC_{50} of 7.25 µL/mL for ABTS and 57.86 µL/mL for scavenging DPPH free radical (21). α-terpineol has potential to reduce melanin, prevent oxidative stress by reducing cellular malondialdehyde, and inhibit tyrosinase that responsible in catalysis of melanin production. Overexpression of melanin induce various dermatologic disorder, so it potent as skin whitening agent (22).

In this research, the antioxidant activity was influenced by the cream base. The antioxidant activity was scavenged optimally, if the active substance can be released from the base and diffuse to the skin tissue. Hydrophilic base has a strong affinity with hydrophilic active substance and provide the highest release of flavonoids as an antioxidants (23,24). Hydrophilic cream less

viscous than hydrophobic or water in oil type of cream, so it can be able to enhance the diffusion of active substance. White champaca methanol extract in cream preparations has a great potential as a natural topical antioxidant, however in this research was limited proven *in vitro*. To understand the impact of formulations on inhibiting skin? aging, further *in vivo* research is necessary. And also, the research of penetration profile of formulations in skin layer is highly recommended, so that white champaca extract can be utilized as an potential ingredient in innovated antioxidant cosmetic products.

Conclusions

The current study indicates that methanol extract of white champaca flower in hydrophilic cream exhibit a very strong activity of antioxidant. It was proven by IC_{50} values of three formulas FI (4%), FII (8%), and FIII (12%) were 29.72, 25.50, and 25.05 µg/ml, respectively. This *in vitro* study indicates that the methanol extract of white champaca flower has a potential as a source of a natural antioxidant.

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Conflict of Interest

The authors have no conflict of interest associated with the material presented in this paper.

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