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SUNSCREEN ACTIVITY DETERMINATION ON *Hippobroma longiflora* LEAVES EXTRACTS AFFECTED TO DIFFERENCES IN SIMPLICIA TREATMENTS AND EXTRACTION TECHNIQUES

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Abstract

Various weeds in paddy rice fields could be utilized as natural polyphenols sources, one of them was kitolod (*Hippobroma longiflora* (L.) G.Don). Kitolod was widely utilized as traditional medicine by several community groups because of its polyphenols content. Recent trends showed kitolod could be valorized as additive matters in sunscreen products. Polyphenols content, especially flavonoids were able to absorb dangerous spectrum in sunray radiation and it is related to sunscreen activity. This study aimed to evaluate the differences in simplicia treatments and extraction techniques towards total polyphenols content and sunscreen activity on kitolod leaf extracts. Solvent-cold extraction techniques were deployed in this study that consist of water infusion and ethanolic maceration for 24 hours at ambient temperature. Extracts were analyzed by spectrophotometric measurement to determine total phenolics content (TPC), total flavonoids content (TFC), and sunscreen activity by deploying Folin-Ciocalteu assay, AlCl3 colorimetric assay, and Mansur approached assay, respectively. Dry kitolod leaves were extracted by ethanolic maceration techniques provided high yields (15.5±0.3%), high TPC (17.9±0.3 mg GAE/g of solids), high TFC (25.9±0.4 mg QE/g of solids), and moderate sunscreen activity (SPF 11.0±0.1). The results indicated kitolod could be valorized as natural polyphenols sources and additive matters in any cosmetical sunscreen products.

INTRODUCTION

The presence of weeds in paddy rice field issued a negative impact on crops in the competition for soil nutrition absorption. Several weeds are considered to have potency value as natural polyphenols sources then supported by their abundance and ability to sprout quickly in nature [1]. Polyphenols have empirical benefits in the food, pharmaceutical and cosmetics, and dye industries because the compounds provided antioxidants, antibacterials, anti-inflammatory, anticarcinogenic, and dyes-coloring properties [2]. Kitolod as weeds was widely utilized in traditional medicine practices by several groups in Indonesia [3], [4]. This utilization emerged because kitolod contains various bioactive metabolites such as polyphenols that affected human well-being.

Recent studies have proven that kitolod has the potential as natural polyphenols source, especially in the leaves. A total of 14 types of polyphenols compounds were identified in kitolod leaves and most of the compounds belong to flavonoid groups [5]. Kitolod leaves were known to have strong antioxidant activity and moderate anticancer activity against HeLa cells [6], [7]. Furthermore, strong antibacterial activities were provided by kitolod leaves against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* [8], [9]. The current trend of kitolod valorization was heading toward the application of additive matters in cosmetic products [10]. Emerged trends existed because kitolod leaves were able to provide ultra protection against sunray radiation with high sunscreen activity, where SPF 28 [11].

Polyphenols content in kitolod leaves was related to the capability of sunscreen activity. Conjugated double-bond in phenolics and keto groups in flavonoids have abilities to absorb and convert sunray radiation into unharmful heat for human skin [12], [13]. Hence, the higher total polyphenols content in natural matters has positive effects on its capabilities to absorb sunray radiation [14]. To obtain high polyphenols content from kitolod leaves, the proper extraction techniques were necessary to be carried out, to obtain extracts with high sunscreen activity.

Cold-solvent extraction techniques were considered proper to be carried out for the extraction and isolation of polyphenols. The thermolabile of polyphenols was the main reason to deploy cold-solvent extraction techniques [15]. Traditional techniques that used solvents and without temperature applied consisted of infusion and maceration techniques [16]. Infusion techniques deployed distilled water as a solvent, while maceration techniques deployed chemical or organic solvents. The type of solvent deployed for extraction should be considered towards the target of compounds and pointed to regulation. Indonesia National Agency of Drugs and Food Control (or BPOM) and the U.S. Food and Drugs Administration (FDA) recommended deploying water and ethanol, a maximum of 70% of concentration, as an extraction solvent for food and cosmetic products which referred to the green chemistry practice [17]. That was a new challenge to conduct extraction in this study to obtain high total polyphenols content and sunscreen activity.

The difference in treatment toward materials or simplicia before conducted extraction process attracted attention to be evaluated in this study. Moisture content in materials, theoretically affected extraction results [18]. This issue is then considered as one of the variables of difference in deployed extraction techniques. This study aimed to evaluate the differences in simplicia treatment and extraction techniques towards total polyphenols content and sunscreen activity on kitolod leaf extracts. The results could be used as literature in the utilization of kitold leaves as valorization for additive matters in cosmetic products with a cost-effective process.

EXPERIMENT

Materials

Kitolod leaves collected from flowering plants (about 3 months old) taken in paddy rice fields on Sukamukti, Majalaya, Bandung. The extraction solvents used were distilled water and 70% of ethanol (technical grade). The analytical materials in this study consist of Folin-Ciocalteu reagent, Na2CO3, AlCl3, ethanol (Merck, Germany), gallic acid, and quercetin for the standard calibration curve (Sigma-Aldrich, USA).

Instrumentations

Instrumentation used in this study consist of Buchi® Multivapor™ P-6, Rotary Evaporator IKA RV10, and Spectrophotometer UV-Vis DLAB® SP-UV1000.

Procedures

Preparation of Simplicia

Preparation of Simplicia as experimental materials followed by Dewantoro *et al.* [5] with additional modification. An additional procedure was conducted for separation at each stage (after washing and drying) to be analyzed its moisture content and stored it as extraction materials. The moisture content analysis procedure deployed gravimetric methods followed by Luliana *et al.* [19]. The simplicity was then labeled as untreated leaves (fresh leaves) and air-dried leaves.

Extraction: Infusion and Maceration

Cold-solvent extraction techniques were deployed consisting of infusion and maceration techniques. The infusion technique followed the procedure from Aritonang *et al.* [20] with modification in time addition to 24 hours to compare untreated leaves and air-drier leaves. Meanwhile, the maceration technique followed Rosidah *et al.* [21] by reducing time to 24 hours toward the highest characterization extracts on infusion technique. The maceration used 70% of ethanol. Supernatants separated from solid residues were then evaporated using multi-vapor and rotary evaporators, respectively, at 50°C until no solvents drips to obtain crude extracts.

Determination of Total Phenolics Content (TPC)

Quantification of total phenolics content (TPC) followed Al-Owaisi *et al.* [22] with slight modification in the wavelength test and incubation time. The maximum absorbance method approach was used to determine the wavelength test and incubation time to be applied in this study [18], [23]. **Figure 1** showed 735 nm of wavelength and 105 minutes of incubation time provide maximum absorbance towards gallic acid standard (100 mg/L). Gallic acid standard calibration curve was made from 0-100 mg/L of concentration as a comparison and provided a standard equation

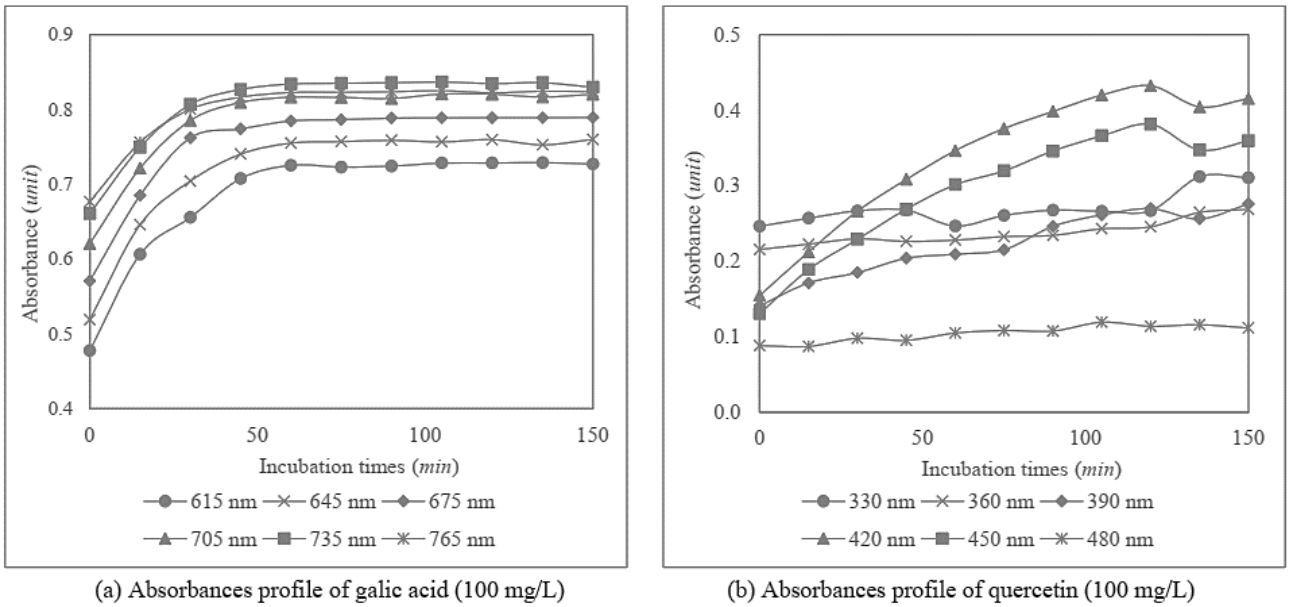
$$y = 0.0081x + 0.0039 \ (R^2 = 0.9916).$$

The measurement value is then substituted to Equation (1).

$$\mathrm{TPC} = \frac{c_P \cdot V \cdot d_f}{m} \qquad \ldots(1)$$

TPC was the result of total phenolics content in *mg GAE/g of solids*; c_P was the concentration of

phenolics in extracts based on the gallic acid calibration equation in *mg/L*; *V* was the volume solvents in *L*; d_f was dilution factors (no unit); and *m* was the total solids weight of Simplicia in *g*.



(a) Absorbances profile of galic acid (100 mg/L) (b) Absorbances profile of quercetin (100 mg/L)

Figure 1. Absorbances profile for (a) gallic acid and (b) quercetin on the determination of wavelength test and incubation time for TPC and TFC procedure, respectively.

Determination of Total Flavonoids Content (TFC)

Quantification of total flavonoids (TFC) was followed by Ningsih *et al.* [24] with slight modification in the wavelength test and incubation time. The maximum absorbance method approach was used and then showed 420 nm of wavelength and 120 minutes of incubation provided the maximum value as well as the absorbance profile in Figure 1. Quercetin standard calibration curve was made from 0-100 mg/L of concentration as comparison and provided standard equation $y = 0.0043x - 0.0174$ $(R^2 = 0.9935)$. The measurement value is then substituted to Equation (2).

$$TPC = \frac{c_F \cdot V \cdot d_f}{m} \qquad \ldots(2)$$

TFC was the result of total phenolics content in *mg QE/g of solids*; C_F was the concentration of flavonoids in extracts based on quercetin calibration equation in *mg/L*; *V* was the volume of the solvent in *L*; d_f was dilution factors (no unit); and *m* was the total solids weight of Simplicia in *g*.

Measurement of Sunscreen Activity

Determination of sunscreen activity was deployed Mansur methods approach followed by Savira & Iskandar [11] and Kusumanti *et al.* [14]. The measured absorbances were then submitted to Equation (3) and quantified activities were expressed as SPF values.

$$SPF = CF \times \sum_{290}^{320} (EE \times I) \times Abs \qquad \ldots(3)$$

SPF was the result of sunscreen activity; CF was the constant of correction factors in the Mansur equation (CF = 10); $EE \times I$ was the spectrum of arrhythmogenic effects and the intensity of sunlight followed by Puspitasari *et al.* [17]; and *Abs* was measured absorbance value.

Statistical Analysis

Collected data were analyzed using t-Test analyses that featured in IBM® SPSS Statistic 26.

RESULT AND DISCUSSION

Physical Properties of Simplicia

Pretreatment aimed to ease the simplicia used in extraction. Drying and size reduction were pretreatment stages that could be deployed toward simplicia. Drying could be reducing moisture content in simplicia that have the potential to accelerate chemical and enzymatic reactions naturally [18]. The presence of water caused inhibition to isolate bioactive metabolites such as polyphenols. Furthermore, the smaller particle size

of simplicia could be increasing surface area affected by the number of bioactive metabolites obtained [25]. **Table 1** showed the results of material weight and moisture content towards simplicia after drying and size reduction treatment.

Table 1. Physical characteristics of simplicial.

Type of Materials	Weight	Moisture Content*
Untreated Simplicia (fresh leaves)	4.500 g	89.8±0.5%
Dry Simplicia (dried leaves)	335 g	9.1±0.3%
Powdered Simplicia (after size reduction stages)	305 g	8.5±0.7%

*Moisture content in wet basis calculation

Moisture content in simplicia has been rated by literature. The regulation for standard moisture content on dry simplicia should be less than 10% (wet basis) according to the Indonesian Ministry of Health [19]. The drying methods deployed were air-drying methods that reduced moisture content by up to 80% for 7 days. Drying time was considered ineffective for application although could maintain bioactive metabolites such as applying an oven blower at 40-60°C to maintain polyphenols from being degraded [26].

Extraction Yields

The differences in material types and extraction techniques descriptively affect extraction results. **Figure 2** showed an infusion of untreated simplicia that have high moisture content provides lower yields than powdered simplicia. The effect of excess moisture content in untreated simplicia proved to have a negative effect because the presence of water in materials inhibited solvent to bind targeted compounds such as polyphenols [18]. The powdered simplicia was then used for maceration to evaluate the effect of different extraction techniques on extraction yields.

The infusion technique towards powdered Simplicia gave lower yields than the maceration technique. Maceration techniques using ethanol as solvent, provided high extraction yield than distilled water on infusion technique because ethanol can bind various components in Simplicia, both polar to nonpolar properties [27]. Meanwhile, distilled water was suspected just able to bind polar components thus providing a low yield [16].

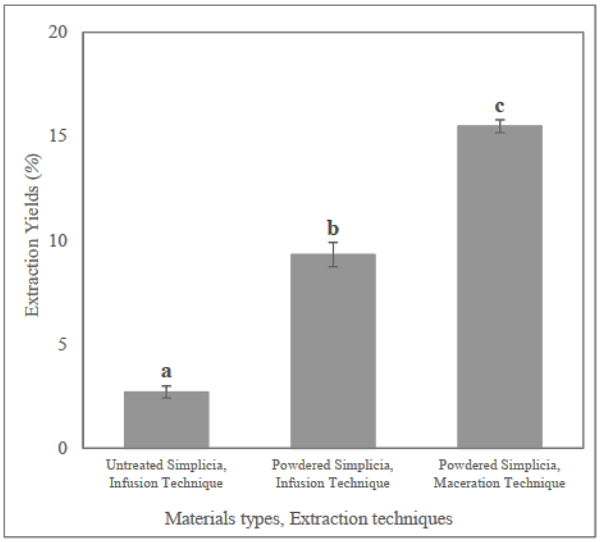


Figure 2. Extraction yields are affected by different Simplicia treatments and extraction techniques. Different symbols indicated significant differences regarded to t-Test assuming equal variances analysis.

Total Polyphenols Content

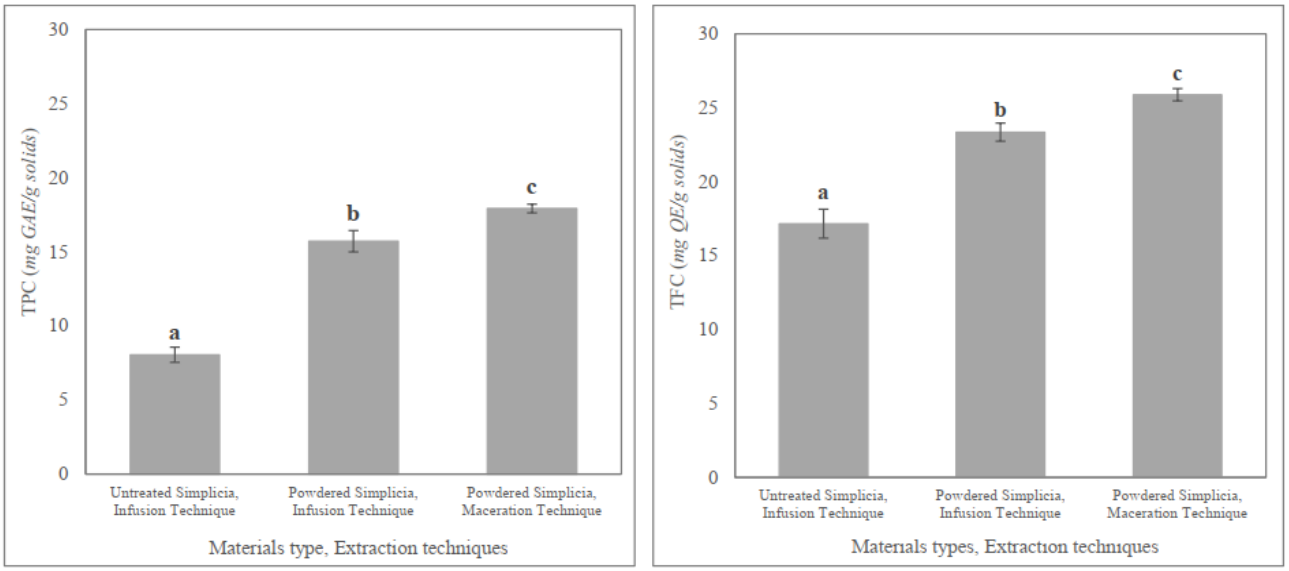
Spectrophotometric measurement methods were deployed to determine total phenolics and flavonoid content. These groups were the most abundant natural polyphenols types, which could be represented by total polyphenols content in simplicia [2], [28]. The results in **Figure 3** showed of the ethanolic maceration technique provided the highest total polyphenols content, both on TPC (17.9±0.3 *mg GAE/g of solids*) and TFC (25.9±0.4 *mg QE/g of solids*), then extracts proceeded by water infusion technique.

Polyphenols belong to compounds with polar properties, regarding compound polarities. The study conducted by Riwanti *et al.* [27] stated that more water contained in ethanol affected the enhancement of compound polarities. It assumed that distilled water in the infusion technique should be able to bind polyphenols in high amounts. However, the extracts proceeded by the ethanolic maceration technique provide the highest total polyphenols content to the infusion technique. The obtained findings indicated polyphenols were not polar, but several polyphenols types belong to semi-polar and can be bonded by ethanol.

ifferences in simplicia treatment affected total polyphenols in extracts. Untreated simplicia provided low total polyphenols content due to their moisture content still being high. The presence of water in materials or simplicia was known to inhibit the binding process of polyphenols by solvent and caused low TPC and TFC obtained [18]. Different results were given by powdered simplicia because of the low level of moisture content. Inhibition of

polyphenols-binding did not occur in dry-powdered simplicia, thus providing high TPC and TFC. Therefore, drying treatment to materials or simplicia is required to be carried out to enhance extraction yields and total polyphenols content.

Measured results of total polyphenols content just showed component that was comparable to standard, which was gallic acid and quercetin. Advanced methods are required to be conducted for compound identification more specifically with the number of components. The recommendation for advanced measurement was to deploy chromatography techniques such as HPLC and or LC-MS to identify polyphenols types and contains percentages.



(a) Total phenolics content (TPC) results (b) Total flavonoids content (TFC) results

Figure 3. The total polyphenols content of kitolod leaves consists of (a) phenolics; and (b) falvonoids. Different symbols indicated significant differences regarded to t-Test assuming equal variances analysis.

Sunscreen Activity

Sunscreen activity provided by extracts was related to their total polyphenols content. The double bonds on polyphenols structure, especially on phenolics groups were able to absorb harmful sunray radiation [12]. In addition, the presence of chromophore units at C-4 of keto on flavonoids was known able to absorb sunray radiation more strongly than phenolics [14]. Then, it could be concluded as the higher the total polyphenols content, the stronger the sunscreen activity obtained. The results are shown in **Figure 4** and the interpreted types of protection on each extract consisted of moderate protection from untreated simplicia extracted by infusion, extra protection from powdered simplicia extracted by infusion, and maximum protection from powdered simplicia extracted by maceration.

The results of this study have a lower protection category and SPF value than previous studies. Savira & Iskandar [11] gained kitolod leaf extracts with ultra-protection of sunscreen activity as SPF 28. However, their results deployed methanol as a solvent which is unrecommended by regulation. Thus, further research on kitolod leaf extraction should be conducted that applied the green chemistry concept and provided high yields nor sunscreen activity.

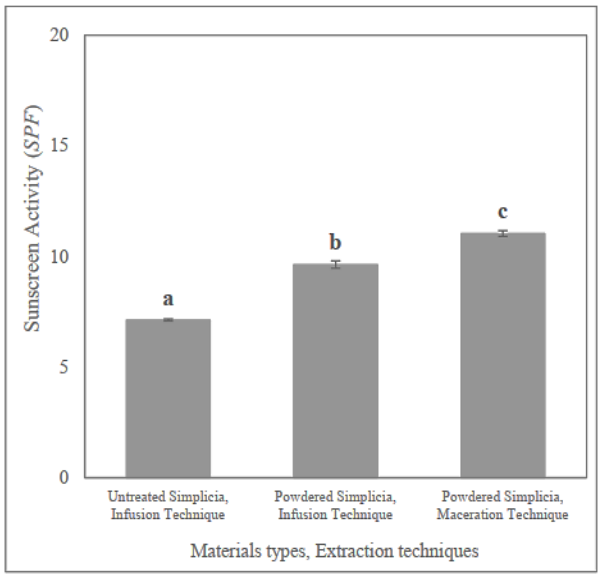


Figure 4. Sunscreen activity of kitolod leaf extracts affected by different simplicia treatments and extraction techniques. Different symbols indicated significant differences regarded to t-Test assuming equal variances analysis.

CONCLUSION

Differences in simplicia treatment and extraction techniques affected extracts. Drying treatment toward simplicia proven enhancing extracts obtained, both in yields, total polyphenols content (TPC and TFC), and sunscreen activity. Ethanolic maceration techniques provided better results than infusion techniques but required to be carried out on further studies to varied ethanol in a lower concentration. Regarded to this study, kitolod leaf extracts are indicated to valorize as natural polyphenols sources and additive matters in any cosmetical sunscreen products.

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REFERENCES

[1] G.R. Egarani, S. Kasmiyati, and E.B.E. Kristiani, "The antioxidant content and activity of various plants organs of kitolod (Isotoma longiflora)", *Biosaintifika*, vol. 12, no. 3, pp. 297–303, 2020, doi: 10.15294/biosaintifika.v12i3.23888.

[2] I. Gutiérrez-del-río *et al.*, "Plant nutraceuticals as antimicrobial agents in food preservation: terpenoids, polyphenols and thiols", *Int. J. Antimicrob. Agents*, vol. 52, pp. 309–315, 2018, doi: 10.1016/j.ijantimicag.2018.04.024.

[3] N.K. Lestaridewi, M. Jamhari, and I. Isnainar, "Kajian Pemanfaatan Tanaman sebagai Obat Tradisional di Desa Tolai Kecamatan Torue Kabupaten Parigi Moutong", *e-JIP BIOL*, vol. 5, no. 2, 2017.

[4] Elfrida, N.S. Tarigan, and A.B. Suwardi, "Ethnobotanical study of medicinal plants used by community in Jambur Labu Village, East Aceh, Indonesia", *Biodiversitas*, vol. 22, no. 7, pp. 2893–2900, 2021, doi: 10.13057/biodiv/d220741.

[5] A.I. Dewantoro, S.H. Putri, and E. Mardawati, "Analisis kualitatif kandungan senyawa polifenol pada daun herba kitolod (Hippobroma longiflora (L.) G.Don) dan potensi pemanfaatannya sebagai sumber polifenol alami", *Agrointek, J. Teknol. Ind. Pertan.*, vol. 16, no. 3, pp. 412–419, 2022, doi: 10.21107/agrointek.v16i3.13235.

[6] N.W. Martiningsih, I.W. Mudianta, and I.A. P. Suryanti, "Phytochemical screening and antioxidant activity of Hippobroma longiflora extracts", *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 1115, 2021, doi: 10.1088/1757-899X/1115/1/012078.

[7] A. Permana *et al.*, "Artikel review: Fitokimia dan farmakologi tumbuhan kitolod (Isotoma longiflora Presi)", *Buana Farma*, vol. 2, no. 3, pp. 22–35, 2022.

[8] H.A. Simanjuntak, "Antibacterial activity of ethhanolic extract of kitolod (Hippobromalongiflora) leaf againts Staphylococcus aureus and Salmonella typhi", *Asian J. Pharm. Res. Dev.*, vol. 8, no. 1, pp. 52–54, 2020, doi: 10.22270/ajprd.v8i1.660.

[9] A.R. Zarta, F. Ariyani, W. Suwinarti, I.W. Kusuma, and E. T. Arung, "Short communication: Identification and evaluation of bioactivity in-forest plants used for medicinal purposes by the Kutai community of East Kalimantan, Indonesia", *Biodiversitas*, vol. 19, no. 1, pp. 253–259, 2018, doi: 10.13057/biodiv/d190134.

[10] A.P. Sabrina, E. Tania, S. Nuryamah, and N. Yuniarsih, "The potential of natural ingredients as sunscreen: A narrative literature review", *Open Access Indones. J. Med. Rev.*, vol. 2, no. 4, pp. 265–269, 2022, doi: 10.37275/oaijmr.v2i4.221 1.

[11] D. Savira and D. Iskandar, "Pemanfaatan ekstrak daun kitolod (Hippobroma longiflora (L) G.Don) sebagai bahan aktif sediaan tabir surya", *J. Kim. Ris.*, vol. 5, no. 1, pp. 44–48, 2020, doi: 10.20473/jkr.v5i1.19680.

[12] S. Saric and R.K. Sivamani, "Polyphenols and sunburn", *Int. J. Mol. Sci.*, vol. 17, no. 9, pp. 1–22, 2016, doi: 10.3390/ijms17091521.

[13] A. Amini, C.D. Hamdin, W.A. Subaidah, and H. Muliasari, "Efektivitas formula krim tabir surya berbahan aktif ekstrak etanol biji wali (Brucea javanica L. Merr)", *J. Kefarmasian Indones.*, vol. 10, no. 1, pp. 50–58, 2020, doi: 10.22435/jki.v10i1.2066.

[14] D.P. Kusumanti, N.A. Sayuti, and I. As, "Aktivitas tabir surya formula bedak dingin jawa", *IJPST*, vol. 1, no. 1, pp. 1–7, 2017, doi: 10.24198/ijpst.v1i1.12156.

[15] M. Fazil, R.N. Suci, F. Allfiah, D.N. Alam, G. Angelia, and B. Situmeang, "Analisis senyawa alkaloid dan flavonoid dari ekstrak kitolod (Isotoma longiflora) dan uji aktivitasnya terhadap bakteri penyebab karies gigi", *J. ITEKIMA*, vol. 2, no. 1, pp. 73–83, 2017.

[16] N.N. Azwanida, "A review on the extraction methods use in medicinal plants, principle, strength and limitation", *Med. Aromat. Plants*,

vol. 4, no. 3, pp. 1–6, 2015, doi: 10.4172/2167-0412.1000196.

[17] A.D. Puspitasari, D.A.K. Mulangsri, and Herlina, "Formulasi krim tabir surya ekstrak etanol daun kersen (Muntingia calabura L.) untuk kesehatan kulit", *Media Penelit. dan Pengemb. Kesehat.*, vol. 28, no. 4, pp. 263–270, 2018, doi: 10.22435/mpk.v288i4.524.

[18] M. Plaza, G. Domínguez-rodríguez, M. Castro-puyana, and M.L. Marina, "Polyphenols analysis and related challenges", in *Polyphenols: Properties, Recovery, and Applications*, C. M. Galanakis, Ed. Elsevier Inc., 2018, pp. 177–232. doi: 10.1016/B978-0-12-813572-3.00006-3.

[19] S. Luliana, N.U. Purwanti, and K.N. Manihuruk, "Pengaruh cara pengeringan simplisia daun senggani (Melastoma malabathricum L.) terhadap aktivitas antioksidan menggunakan metode DPPH (2,2-difenil-1-pikrilhidrazil)", *Pharm. Sci. Res.*, vol. 3, no. 3, pp. 120–129, 2016.

[20] H.F. Aritonang, H. Koleangan, and A.D. Wuntu, "Synthesis of silver nanoparticles using aqueous extract of medicinal plants' (Impatiens balsamina and Lantana camara) fresh leaves and analysis of antimicrobial activity", *Int. J. Microbiol.*, vol. 2019, pp. 1–8, 2019, doi: 10.1155/2019/8642303.

[21] A.N. Rosidah, P.E. Lestari, and P. Astuti, "Daya antibakteri ekstrak daun kendali (Hippobrima longiflora [L] G. Don) terhadap pertumbuhan Streptococcus mutans", *Artik. Ilm. Has. Penelit. Mhs.*, 2014.

[22] M. Al-Owaisi, N. Al-Hadiwi, and S.A. Khan, "GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves", *Asian Pac. J. Trop. Biomed.*, vol. 4, no. 12, pp. 964–970, 2014, doi: 10.12980/APJTB.4.201414B295.

[23] A. Blainski, G.C. Lopes, and J.C.P. de Mello, "Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L.", *Molecules*, vol. 18, pp. 6852–6864, 2013, doi: 10.3390/molecules18066852.

[24] I.Y. Ningsih, S. Zulaikhah, M.A. Hidayat, and B. Kuswandi, "Antioxidant activity of various kenitu (Chrysophyllum cainito L.) leaves extracts from Jember, Indonesia", *Agric. Agric. Sci. Procedia*, vol. 9, pp. 378–385, 2016, doi: 10.1016/j.aaspro.2016.02.153.

[25] E.S. Prasedya *et al.*, "Effect of particle size on phytochemical composition and antioxidant properties of Sargassum cristaefolium ethanol extract", *Sci. Rep.*, vol. 11, no. 1, pp. 1–9, 2021, doi: 10.1038/s41598-021-95769-y.

[26] I. Hasbay and C.M. Galanakis, "Recovery technologies and encapsulation techniques", in *Polyphenols: Properties, Recovery, and Applications*, C. Galanakis, Ed. Elsevier Inc., 2018, pp. 233–264. doi: 10.1016/B978-0-12-813572-3.00007-5.

[27] P. Riwanti, F. Izazih, and Amaliyah, "Pengaruh perbedaan konsentrasi etanol pada kadar flavonoid total ekstrak etanol 50, 70 dan 96% Sargassum polycystum dari Madura", *J. Pharm. Care Anwar Med.*, vol. 2, no. 2, pp. 35–48, 2020, doi: 10.36932/jpcam.v2i2.1.

[28] A.N. Panche, A.D. Diwan, and S.R. Chandra, "Flavonoids: an overview", *J. Nutr. Sci.*, vol. 5, pp. 1–15, 2016, doi: 10.1017/jns.2016.41.