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SERUM OF EXTRACT *Onchidium Typhae* USES HYDROXYETHYL CELLULOSE AND CARBOXYMETHYL CELLULOSE NATRIUM BASE AS ANTIOXIDANT

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ABSTRACT

Antioxidants can prevent premature aging (anti-aging) and various types of cancer and increase body immunity. Empirically, Onchidiid slugs have been used as natural antioxidants. This study aimed to determine the antioxidant potency of the serum of Onchidiid slug ethanolic extract. The Onchidiid slug extract was obtained by maceration and was prepared to create two effective serum formulations. Formula A involved hydroxyethyl cellulose (HEC), while Formula B employed sodium carboxymethyl cellulose (Na-CMC) as gelling agent. The second serum formula was rigorously evaluated and subjected to antioxidant activity testing by the diphenyl-2-picrylhydrazyl (DPPH) method. The serum preparation with the best physical properties and antioxidant activity was serum A at a concentration of 1%. Physical test results showed that serum A had a yellow color with a distinctive smell and was homogeneous with a soft texture. The test results for the spreadability and stickiness properties of serum A were 6 cm^2 and 1'55" second. The serum A antioxidant activity test results showed moderate free-radical scavenging activity, with an IC_{50} value of 277 ppm.

Keywords: Anti-aging, 2,2-diphenyl-1-pikrilhidrazyl, *Onchidium typhae*, Antioxidant

INTRODUCTION

Indonesia consists of many islands, and is home to various types of plants and animals. Their marine resources are diverse and abundant. One potential resource is onchidiid slugs, known locally as "Lintah Babi" which contain natural antioxidants to help prevent premature aging and cancer (von Rintelen et al., 2017). In addition to being a food source, the exploitation of marine resources can also yield bioactive compounds with significant economic value. Onchidiid slugs, which are shell-less, are commonly found in the waters of West Kalimantan. The scientific name of The onchidiid slug species is *Onchidium typhae* **Figure 1**, which thrives in brackish waters (Wang et al., 2021; Wijianto et al., 2022a, 2022b).

Figure 1. The Appearance Of *Onchidium typhae*

The type of damage to the skin, such as rough skin, dull skin, dry skin, hard skin, cracked skin, cracked black spots, and wrinkles, is a part of the self's response to protect the body's skin to protect itself from external influences. Skin damage can occur because of oxidative stress in the body caused by free radicals (Pandanwangi et al., 2018; Poljšak and Dahmane, 2012).

Sources of free radicals can be obtained from outside the body (external) and inside the body (internal). Examples of free radicals from outside the body (external) include pollution, smoking, ozone, UV rays, pesticides, radiation, and chemicals. The human body produces free radicals through metabolic processes, such as inflammation, xanthine oxidase, phagocytes, peroxisomes, and the arachidonic pathway (Phaniendra et al., 2015). Excessive levels of free radicals can trigger diseases and degenerative conditions, such as premature aging, wrinkles, and skin cancer (Caiati et al., 2023). Antioxidants can counteract free radicals by binding to them and inhibiting the cell damage caused by oxidation reactions (Lobo et al., 2010).

Antioxidants protect the body by stabilizing free radicals and safeguarding proteins, tissues, cells, and organs (Chaudhary et al., 2023). Antioxidants can prevent premature aging (anti-aging), heart disease, blindness, and various types of cancer and can increase human immunity. Onchidiid slugs are potent natural antioxidants used for various purposes. Based on previous research, the onchidiid slug extract had an IC_{50} of 92.045 ppm, which is categorized as strong (Kemuning et al., 2022). Onchidiid slugs contain phytochemical compounds such as alkaloids and steroids, which are responsible for their antioxidant activity. In addition to their antioxidant properties, onchidiid slugs (*Onchidium thypae*) have potential use in wound medicine. (Wijianto & Hamzah, 2022).

There has been no research on the antioxidant activity of onchidiid slug extract in serum preparations. Serum with a cellulose base can form a serum consistency that is comfortable to use on the skin because it can speed up absorption into the skin and maintain a healthy skin. Serum products are popular among teenagers and adults because they are affordable (Usman et al., 2023). Serum was contituted from active ingredients and gel base. This base is important for achieving the desired consistency and stability. Several previous studies inspired this study. Serum from robusta green coffee extract (*Coffea canephora var. Robusta*) as an antioxidant with the gelling agent hydroxyethyl cellulose (HEC) has been studied, and serum preparations with a pH in the range of 4.34-4.51 and spreadability in the range of 5.55-5.70 cm (Mardhiani et al., 2018). The semisynthetic gel base is not easy for microbes to grow, thus affecting comfortable cold conditions (Kumar et al., 2012). Based on SNI 16-4380-1996, the safe cosmetic pH is in the skin pH range of 4.5 to 6.5. An optimization study using a CMC-Na gelling agent significantly impacted the pH value. In contrast, an increase in CMC-Na composition has a notable effect on increasing viscosity (Ningtyas, 2020). CMC-Na is a cellulose derivative used as a gel base with a stable viscosity, resistance to microbial growth, clear gel base, and strong film on the skin when applied (Adnan, 2016). CMC-Na is used as a gelling agent because it is easy to mix with active substances, and the resulting gel is more transparent (Widyaningrum et al., 2019).

The use of natural ingredients in topical preparations is relatively safe, inexpensive, easy to use, and obtained from renewable sources. This study aimed to determine the antioxidant activity of the ethanolic extract of onchidiid slugs that were incorporated into serum preparations.

RESEARCH METHOD

Tools and materials

The tools used in this research include Rotary evaporator (Buchi R-i100 9230 Flawil)); glassware (Pyrex); analytical balances (Ohaus); Micropipette (Eppendorf), UV/Vis Spectrophotometer (Shimadzu UV-2450), hotplate (Lab Companion, oven (Modena). Materials used in this study include onchidiid slug fresh meat, Sodium Carboxymethyl Cellulose (Wealthy), DMDM Hydantoin (Purenso Select), glycerin (Ashwin Pharma), propylene glycol (Melbourne Solvent), rose oil (Aloin), Hydroxy Ethyl Cellulose (Arshine

Group), Phenoxyethanol (Multi Kimia), Ethylenediaminetetraacetic acid (Merck), 2,2-Diphenyl-1-picrylhydrazyl (Sigma).

The course of the research

This research was carried out systematically, encompassing several stages of experimentation. The first stage involved collecting onchidiid slug samples. Next, the slugs were processed to extract their powder, which was used to create an ethanol extract. In the third stage, serum formulas were prepared using onchidiid slug ethanol extract. These serum formulas were subjected to physical evaluation to ensure physical stability. Finally, serum preparations were tested for antioxidant activity to determine their effectiveness against oxidative stress. The process was conducted with utmost care and precision, to ensure accurate and reliable results.

1. **Preparation of Onchidiid slug powder**

   Before the research, the samples were tested at the FMIPA Biology Laboratory of the Universitas Tanjungpura, and the research was conducted ethically, as approved under No.1653/UN22.9/PG/2022. The flesh of the onchidiid slugs was cleaned and separated from mucus. Drying of the flesh was required to reduce the water content (48 hours at 60-70°C). The dried sample was pulverized prior to extraction (Wijianto et al., 2022a).

2. **Onchidiid slug ethanolic extract preparation**

   The dry powder was weighed and macerated by soaking the sample in 100 mL of ethanol solvent, covering it, storing it in a glass beaker, and occasionally stirring it until the solvent was serene. Next, the filtrate was extracted using a Buchner funnel. Evaporate extraction results at 70ºC and 70 rpm (Wijianto et al., 2022a). After the extract was obtained, the next step was to prepare serum using several formulas, as shown in **Table I** and

Table II**.**

3. Serum Formulation

Table I. Serum Formulation A Of Onchidiid Slug Ethanolic Extract

Material	Concentration (%)		
	F1	**F2**	**F3**
Ethanol extract of Onchidiid slug	0.5	1	3
Hydroxy Ethyl Cellulose	1	1	1
Glyserin	10	10	10
Phenoxyethanol	0.5	0.5	0.5
Na_2EDTA	0.05	0.05	0.05
Aquadest	ad 100	ad 100	ad 100

Preparation of serum formula A (variation of ethanol extract of onchidiid slug concentration and use of HEC as a gelling agent) was initiated by dispersing hydroxyethyl cellulose using hot water at 80 °C, adding glycerin, and stirring until homogeneous. Subsequently, Na_2EDTA and phenoxyethanol were added, and the ethanol extract of the onchidiid slugs was added until a suitable serum preparation was formed.

Table II. Serum Formulation B Of Onchidiid Slug Ethanol Extract

Materials	Concentration (%)		
	F1	F2	F3
Ethanolic extract of *Onchidiid* slug	0.5	1	3
Na CMC	4	4	4
DMDM Hydantoin	0.5	0.5	0.5
Glyserin	10	10	10
Propylene glycol	2	2	2
Rose oil	3 drops	3 drops	3 drops

Preparation of serum formula B (variation of ethanol extract of *onchidiid* slug concentration and use Na CMC as gelling agent). F1, F2, and F3 are serum samples with extract concentrations of 0.5%, 1%, and 3%, respectively. Begins by first developing Na-CMC using hot water at 80 °C DMDM hydantoin and propylene glycol were dissolved, added to expanded CMC Na, and crushed until homogeneous. Onchidiid slug extract at each concentration was added, followed by the addition of water and rosae oil. Stir until a homogeneous and suitable serum gel mass is formed.

Using HEC and CMC-Na as gelling agents at different extract concentrations can affect their antioxidant activity. Previous studies have shown that 1% HEC serum base and 4% CMC-Na produced serum preparations that met physical requirements. So, it is necessary to test the effectiveness of antioxidants with varying extract concentrations (Mardhiani dkk, 2018; Widyaningrum et al., 2019)

4. Physical evaluation of serum onchidiid slug ethanol extract

a. Organoleptic test

The serum was tested organoleptically by evaluating its texture, color, and odor. The organoleptic test aims to determine the physical condition of the serum preparation (Tilarso, 2022).

b. pH test

The pH test was carried out by calibrating the pH meter using pH seven and four buffers. The pH meter was alternately dipped and then dried to ensure that it was completely dry. The next step was to dip the pH meter into serum. The pH test requirements were 4.5 to 6.5 (Tilarso, 2022).

c. Spreadability test

Spreadability testing was performed by weighing serum (0.5 g) and placing it in the center of a round glass with a scale. Then, another round of glass was placed on the top, left for 1 minute and added to a weight. The spreadability requirement that meets the requirements is a diameter of 5-7 cm (Tilarso, 2022).

d. Adhesion test

A total of 0.5 Â g of the preparation was placed between two glass objects and then weighed 1 kg within 5 minutes. The time required to separate the two glass objects is determined. An ideal adhesion test requires more than 4 seconds (Tilarso, 2022).

5. Measurement of serum antioxidant activity

Determination of the IC_{50} value was carried out using serum with the optimal formulation. Various concentrations were prepared, covering 100, 200, 300, 400, 500, 600, and 700 ppm. In the next step, 3 mL of each sample was pipetted, 3 mL of DPPH 39.4 ppm was added, and the mixture was incubated in a closed room at 37°C for 30 minutes. The absorption of the solution was measured using a UV/Vis spectrophotometry (515,5 nm). Replication was performed three times.

Data Analysis

Data analysis used the linear regression formula y = bx+a. In the linear regression calculation data, y is the percent inhibition value (antioxidant) and x is the extract concentration. Antioxidant activity was determined by the amount of inhibition of DPPH radical absorption by calculating the percent inhibition of DPPH absorption using the formula below:

$$\%\text{DPPH activity} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100\%$$

RESULTS AND DISCUSSION

Results of Physical Evaluation of Serum Preparations

The physical evaluation results of Formulas A and B are listed in **Table III.** From the results of the organoleptic test, it is known that both formulas A and B form a thick serum preparation with a yellow color, as shown in **Figure 2**, with a typical smell of a slug.

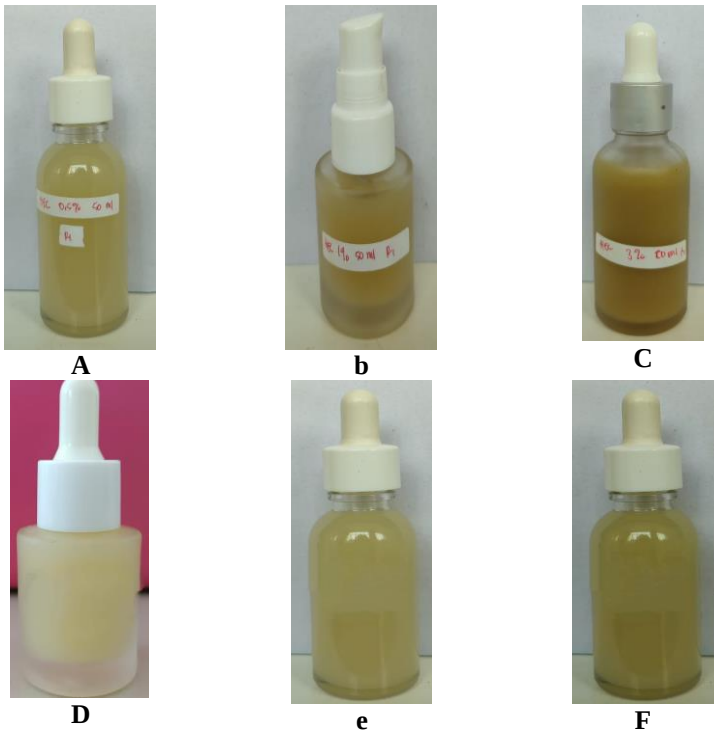

Figure 2. The appearance of serum preparations with different gel bases (Hydroxyethyl Cellulose: A,B And C And CMC-Na: D, E And F) and extract concentrations (A and D: 0.5%; B and E: 1%; C and F: 3%).

The preparation of the onchidiid slug extract in this study was carried out using the maceration method for ± 72 hours with 96% ethanol. The percentage yield of onchidiid slug extract obtained was 7.1%. The results of tests for homogeneity, pH, spreadability, and adhesiveness of the serum can be seen in **Table III.**

Table III. Physical Evaluation Result Of Serum

Formula Serum	Extract concentration (%)	Test Result			
		Homogeneity	pH	Spreadability (cm)	Adhesion (sec)
A	0.5	Homogen	5.4	6.7	66
	1	Homogen	5.5	6.0	115
	3	Homogen	5.6	5.8	148
B	0.5	Homogen	5.7	7.6	67
	1	Homogen	5.9	7.1	71
	3	Homogen	5.9	7.0	86

The evaluation results of onchidiid slug extract serum preparations in formulas A and B (**Figure 2**) appeared homogeneous because there were no solid substances or lumps. The pH values of serum formulations A and B at concentrations of 0.5%, 1%, and 3% met the pH requirements for topical skin preparations, namely 4.5-6.5. The pH values in serum A and serum B were not significantly different. If the pH value of the preparation is too low, it can irritate the skin, whereas if the pH value of the preparation is too high, it can cause the skin to become dry and scaly (Pandanwangi et al., 2018). In testing the spreadability of serum preparations, it was seen that the diameter of the preparation spread decreased along with increasing variations in the concentration of onchidiid slug ethanol extract. The spreadability requirement for topical preparations is 5-7 cm, so the spreadability value of the serum formula can still be accepted by the skin (Rumanti et al., 2023; Usman et al., 2023). Spreadability can affect the ability of the serum preparation to spread on the skin, where the higher the spreadability value, the easier it will be for the preparation to spread (Hikmah et al., 2023). Next is adhesion testing; the requirements for a good adhesion test on topical preparations are more than 4 seconds, so chided serum does not meet the adhesion test requirements because they range from 1 to 2 seconds (Pandanwangi et al., 2018). The gelling agents HEC and CMC-Na can increase the adhesive ability and viscosity of the serum structure network. Higher drug absorption is possible with longer serum contact time on the skin. HEC and CMC-Na has a sticking capacity of more than one second (Binder et al., 2019; Li et al., 2021; Putri Rahmani and Zulkarnain, 2023).

Result of Antioxidant test serum

The second serum (concentration 0.5%, 1%, and 3%) was tested for antioxidant activity. In determining the maximum wavelength of DPPH, the maximum wavelength was obtained at 514 nm, so this wavelength was used to test the antioxidant activity of serum. The absorbance, % inhibition, and IC_{50} values can be seen in **Table III**. To get the linear equation y = bx + a, measure concentration against % inhibition in Microsoft Excel and calculate the IC_{50} value for each formula. IC_{50} measures antioxidant activity by determining the concentration of antioxidants that can inhibit 50% of DPPH free radical activity. (Pandanwangi et al., 2018).

Previous research shows that onchidiid snail extract has an antioxidant activity of 92.045 ppm, a strong antioxidant category. (Kemuning et al., 2022). However, after formulation into a serum preparation, the antioxidant activity of the preparation decreased. It was assumed that the storage duration and conditions may cause a decrease in antioxidant activity in the serum preparations tested.

Table IV. Result Of Antioxidant Activity Serum

Sample	Formula	IC_{50} (ppm)
Formula A	F1	900
	F2	277
	F3	3685
Formula B	F1	9166.67
	F2	3947.5
	F3	12435

Formula A: F1: serum A onchidiid slug ethanol extract 0,5%, F2: serum A onchidiid slug ethanol extract 1%, F3: serum A onchidiid slug ethanol extract 3%
Formula B: F1: serum B onchidiid slug ethanol extract 0,5%, F2: serum B onchidiid slug ethanol extract 1%, F3: serum B onchidiid slug ethanol extract 3%

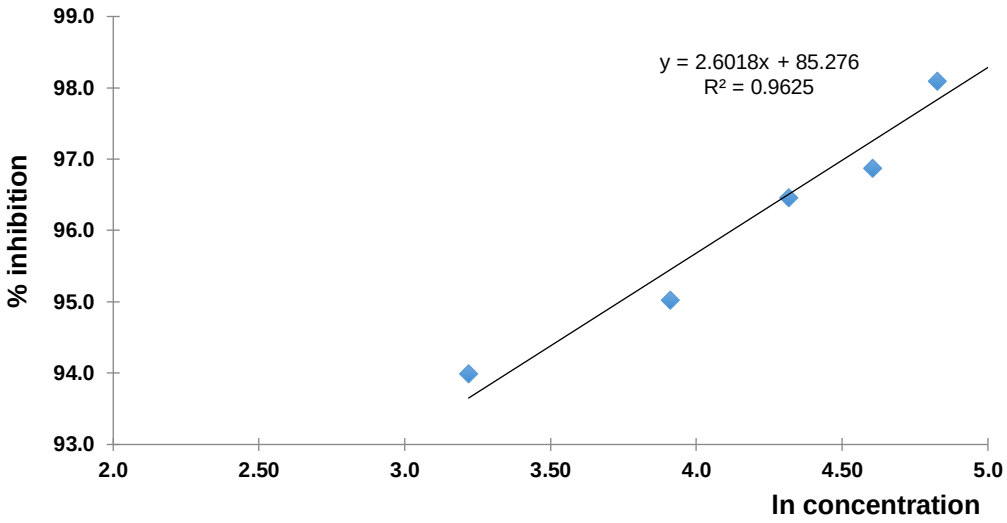

Figure 3. Linear relationship between % inhibitory activity of onychidiid ethanolic extract and the related natural logarithm value of concentration.

An IC_{50} value of 101-150 ppm indicates moderate antioxidant activity, while an IC_{50} value of 50-100 ppm indicates strong antioxidant activity. The smaller the IC_{50} value of a compound being tested, the higher its antioxidant activity (Pandanwangi et al., 2018).

CONCLUSION

This study formulated and evaluated serum preparations using Onchidium typhae extract (obtained from a type of sea slug) with two different ointment bases. The results showed that Formulas A and B met the requirements for organoleptic, homogeneity, spreadability, and pH tests. Serum extract formula A, containing 1% ethanol and HEC base, displayed moderate antioxidant activity with an IC_{50} value of 277 ppm.

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REFERENCES

Adnan, J., 2016. Formulasi Gel Ekstrak Daun Beluntas (Pluceaindica Less) dengan Na-CMC sebagai Basis Gel. J. Pharm. Sci. Herb. Technol. 1, 41–44.

Binder, L., Mazál, J., Petz, R., Klang, V., Valenta, C., 2019. The role of viscosity on skin penetration from cellulose ether-based hydrogels. Skin Res. Technol. 25, 725–734. https://doi.org/10.1111/srt.12709

Caiati, C., Stanca, A., Lepera, M.E., 2023. Free Radicals and Obesity-Related Chronic Inflammation Contrasted by Antioxidants: A New Perspective in Coronary Artery Disease. Metabolites 13. https://doi.org/10.3390/metabo13060712

Chaudhary, P., Janmeda, P., Docea, A.O., Yeskaliyeva, B., Abdull Razis, A.F., Modu, B., Calina, D., Sharifi-Rad, J., 2023. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front. Chem. 11. https://doi.org/10.3389/fchem.2023.1158198

Hikmah, F.N., Malahayati, S., Nugraha, D.F., 2023. Formulasi Dan Evaluasi Sediaan Serum Gel Ekstrak Bunga Melati (Jasminum sambac L.): Formulasi Dan Evaluasi Sediaan Serum Gel Ekstrak Bunga Melati (Jasminum sambac L.). J. Pharm. Care Sci. 3, 93–108. https://doi.org/10.33859/jpcs.v3i2.248

Kemuning, G.I., Wijianto, B., Fahrurroji, A., 2022. Uji Antioksidan Ekstrak Metanol Siput Onchidiid (Onchidium Typhae) Dengan Metode Dpph. J. Ilmu Kedokt. Dan Kesehat. Indones. 2, 130–139.

Kumar, S.N., Vijaybhaskar, D., Rao, P.K., Pratima, S., 2012. Pathological observations on the treatment of oral sub mucous fibrosis of curcumin gels in animal models. Pharm. Lett. 4, 919–926.

Li, X., Deng, Q., Wang, S., Li, Q., Zhao, W., Lin, B., Luo, Y., Zhang, X., 2021. Hydroxyethyl Cellulose As a Rheological Additive for Tuning the Extrusion Printability and Scaffold Properties. 3D Print. Addit. Manuf. 8, 87–98. https://doi.org/10.1089/3dp.2020.0167

Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn. Rev. 4, 118. https://doi.org/10.4103/0973-7847.70902

Mardhiani, Y.D., Yulianti, H., Azhary, D., Rusdiana, T., 2018. Formulasi dan Stabiltas Sediaan Serum dari Ekstrak Kopi Hijau (Coffe canephora var. Robusta) sebagai Antioksidan. Indones Nat Res Pharm J 2, 19–33. https://doi.org/doi.org/10.52447/inspj.v2i2.910

Ningtyas, A.P.W., 2020. Optimasi Hydroxypropyl Methylcellulose dan Carboxymethylcellulose Sodium Dalam Masker Gel Peel Off Antioksidan Ekstrak Daun Jamblang (Syzygium cumini L.) (Skripsi). Universitas Jember, Jember.

Pandanwangi, S., Bachtiar, A., Firmansyah, D., 2018. Uji Aktivitas Antioksidan Krim Kombinasi Ekstrak Daun Jambu Biji (Psidium guajava L.) Dan Ekstrak Umbi Wortel (Daucus carota L.) Dengan Menggunakan Metode DPPH (2,2-diphenyl-1-picrylhydrazyl). Med. Sains J. Ilm. Kefarmasian 3, 31–42. https://doi.org/10.37874/ms.v3i1.62

Phaniendra, A., Jestadi, D.B., Periyasamy, L., 2015. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J. Clin. Biochem. 30, 11–26. https://doi.org/10.1007/s12291-014-0446-0

Poljšak, B., Dahmane, R., 2012. Free Radicals and Extrinsic Skin Aging. Dermatol. Res. Pract. 2012, 1–4. https://doi.org/10.1155/2012/135206

Putri Rahmani, S.I., Zulkarnain, A.K., 2023. Optimization of HPMC and Na-CMC as Gelling Agents on Physical Properties and Stability in Sunflower Seed Oil Gel Formulation. J. Food Pharm. Sci. 812–819. https://doi.org/10.22146/jfps.8227

Rumanti, R.M., Handayani, S., Khairani, T.N., Hafiz, I., 2023. Evaluasi dan Potensi Daya Hambat Gel Antiseptik Tangan Ekstrak Etanol Daun Kemangi (Ocimum sanctum L.) terhadap Bakteri Patogen Pseudomonas aeruginosa dan Staphylococcus epidermidis. J. Pharmascience 10, 23. https://doi.org/10.20527/jps.v10i1.13490

Tilarso, D., 2022. Pengaruh Gelling Agent Pada Sediaan Serum Jerawat Kombinasi Ekstrak Daun Sirih Hijau Dan Buah Belimbing Wuluh. J. AFAMEDIS 3, 1–7. https://doi.org/10.61609/afamedis.v3i1.45

Usman, H.N., Pratiwi, L., Wijianto, B., 2023. Cosmetic Serum Loaded Arabica Coffee (Coffea arabica) Extract: Formulation and Antioxidant Study. Tradit. Med. J. 8, 93–101. https://doi.org/10.22146/mot.83120

von Rintelen, K., Arida, E., Häuser, C., 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. Res. Ideas Outcomes 3, e20860. https://doi.org/10.3897/rio.3.e20860

Wang, B., Chen, D., Yu, M., Liu, Y., Liu, P., Zhang, X., 2021. A Review on Metabolites from Onchidium Genus: Chemistry and Bioactivity. Chem. Biodivers. 18, e2000580. https://doi.org/10.1002/cbdv.202000580

Widyaningrum, N., Novitasari, M., Puspitasari, K., 2019. Perbedaan Variasi Formula Basis CMC Na terhadap Sifat Fisik Gel Ekstrak Etanol Kulit Kacang Tanah (Arachis hypogaea L). Avicenna J. Health Res. 2, 121–134. https://doi.org/doi: 10.36419/avicenna.v2i2.308.

Wijianto, B., Hamzah, H., 2022. Efficacy of onchidiid slug (onchidiium typhae) ethanolic extract against bacterial and fungal grown in biofilm cultures. Eur. Chem. Bull. 11, 112–116. https://doi.org/10.31838/ecb/2022.11.10.015

Wijianto, B., Hamzah, H., Nurhidayah, A.L., Kemuning, G.I., Dyas, R.A.A., 2022a. Characterization of Onchidiid Slug (Onchidium typhae) West Kalimantan Waters as

Antibacterials and Antifungal. Borneo J. Pharm. 5, 35–41. https://doi.org/10.33084/bjop.v5i1.2936

Wijianto, B., Nurhidayah, A.L., Luliana, S., 2022b. Standardization of secondary metabolites and heavy metal contamination assay on onchidiid slug (Onchidium typhae) West Kalimantan waters. J. Farm. Sains Dan Prakt. 8, 199–206. https://doi.org/10.31603/pharmacy.v8i3.7296