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Antifungal activity of shampoo formulated with water spinach *(Ipomoea aquatica)* ethanolic extract against *Pityrosporum ovale*

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ABSTRACT

Background: Dandruff, primarily caused by *Pityrosporum ovale*, is a common scalp disorder, especially in tropical regions. Natural alternatives, such as water spinach (*Ipomoea aquatica*), are increasingly being explored for their antimicrobial properties due to their minimal side effects.

Objective: This study aimed to formulate an anti-dandruff shampoo using ethanol extract of water spinach and evaluate its physical properties and antifungal activity against *P. ovale*.

Results: The phytochemical screening of the water spinach extract confirmed the presence of polyphenols, flavonoids, and quinones, known for their antimicrobial properties. The shampoo formulations met pH and foam height standards and remained organoleptically stable over 28 days. However, viscosity decreased below the standard (<400 cps) after 28 days. The 4% extract formulation (F3) showed the highest antifungal activity with an inhibition zone of 10.01 mm, significantly greater than the negative control ($p<0.05$).

Conclusion: Water spinach ethanol extract can be successfully formulated into a shampoo that meets most physical requirements and exhibits antifungal activity against *P. ovale*. The 4% extract formulation demonstrated the highest effectiveness, though viscosity stability requires further optimization.

Keywords: *Ipomoea aquatica*, antifungal, shampoo, *Pityrosporum ovale*, natural extract

Introduction

Dandruff is a common scalp issue, particularly in tropical and high-temperature regions such as Indonesia. It is a mild scalp disorder characterized by inflammation, sometimes accompanied by itching and hair loss [1]. The condition may be caused by various factors, including excessive secretion from sebaceous glands or the presence of microorganisms on the scalp [2]. One such microorganism is *Pityrosporum ovale* (also known as *Malassezia*), a fungus that typically constitutes less than 47% of the scalp's microbial population under normal conditions. However, certain trigger factors can disturb the balance of the scalp's flora, leading to an overgrowth of *P. ovale* and the subsequent development of dandruff [3].

With advancements in medicine, natural ingredients are increasingly being explored as safer alternatives to chemical treatments due to their minimal side effects. One such natural remedy is water spinach (*Ipomoea aquatica Forsk.*), which is rich in minerals, nutrients, and secondary metabolites such as flavonoids, polyphenols, alkaloids, tannins, saponins, and quinones. These compounds have demonstrated antimicrobial properties and potential use as natural antibiotics [4]. Previous studies have shown that water spinach extract at a concentration of 3.13% is effective in inhibiting the growth of *P. ovale*, comparable to 1% ketoconazole in vitro [5].

Based on these findings, this study aims to explore the use of ethanol extract from water spinach as an anti-dandruff agent by formulating it into a shampoo and evaluating its activity against *P. ovale*, the fungus responsible for dandruff.

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Table 1. Anti-dandruff shampoo formula

Ingredient	Formulation of anti-dandruff shampoo with various concentrations of water spinach ethanol extract				
	NC	F1	F2	F3	PC
Water spinach extract	0 %	2 %	3 %	4 %	Brand X shampoo 1%
Sodium lauryl sulfate	10 %	10 %	10 %	10 %	
Cocamide DEA	4 %	4 %	4 %	4 %	
Na CMC	3 %	3 %	3 %	3 %	
Methylparaben	0.15 %	0.15 %	0.15 %	0.15 %	
Citric acid	qs	qs	qs	qs	
Menthol	0.5	0.5	0.5	0.5	
Aqua	ad 30ml	ad 30ml	ad 30ml	ad 30ml	

Source: Malonda *dkk,* 2017 [7]

Method

Plant identification

The identification of the water spinach (*Ipomoea aquatica Forsk.*) used in this study was conducted at the Plant Taxonomy Laboratory, Faculty of Biology, Jenderal Soedirman University. The plant material was obtained from Dukuhwaluh village, Purwokerto, and confirmed as belonging to the *Ipomoea aquatica* species, part of the Convolvulaceae family, using references from the *Flora of Java* library.

Preparation of simplicia and extract

The preparation of simplicia began by thoroughly washing the plant material with tap water. The leaves and young stems of the water spinach were then separated and reduced in size. The plant material was dried under the sun, covered with a black cloth to minimize compound degradation due to oxidation. Drying was considered complete when the material crumbled easily upon pressure. The dried water spinach was then powdered using a blender, and 200 g of the powder was subjected to extraction using the maceration method with 2 liters of 70% ethanol. The maceration process lasted three days, with occasional stirring. Afterward, the liquid extract was filtered, and the ethanol solvent was evaporated using a rotary vacuum evaporator (IKA) until a thick extract was obtained. The resulting extract was weighed and stored in a sealed glass container until further testing.

Phytochemical screening

Phytochemical screening was performed to detect polyphenolic, flavonoid, and quinone compounds in the ethanol extract of water spinach using wet reaction methods [6].

- Polyphenols: A small amount of powdered simplicia was heated in a water bath and filtered. The filtrate was treated with iron (III) chloride reagent, and the presence of polyphenols was indicated by a green-blue to black coloration.
- Flavonoids: Powdered simplicia was mixed with magnesium powder and 2 N hydrochloric acid, heated, and filtered. Amyl alcohol was then added to the filtrate, and the mixture was shaken. A yellow to red coloration in the amyl alcohol layer indicated the presence of flavonoids.
- Quinones: Powdered simplicia was heated in a water bath, and the filtrate was treated with 5% potassium hydroxide solution. The presence of quinones was indicated by a yellow to red color.

Shampoo formulation

The shampoo was formulated using water spinach ethanol extract at concentrations of 2%, 3%, and 4%, along with other excipients (Table 1). The process began by dissolving methylparaben (Golden Era) in hot distilled water, followed by the gradual addition of sodium lauryl sulfate (Spectrum), cocamide DEA (IMJ), and Na CMC (Sigma). After cooling, the water spinach extract, pre-dissolved in distilled water, was incorporated into the mixture. Citric acid (Emsure) was added to adjust the pH, and menthol was included for fragrance.

Evaluation of shampoo physical properties

The shampoo formulations were evaluated for organoleptic properties (appearance, color, and odor),

Table 2. Phytochemical screening results

Compound class	Reagent	Results	
		Color	Intepretation
Polyphenolates	$FeCl_3$	Blackish green	+
Flavonoid	Mg and HCl 2N	Yellow	+
Quinones	KOH 5%	Deep yellow	+

pH, viscosity, and foam height. Organoleptic properties were observed at room temperature (28–30°C). The pH was measured using pH indicator paper, and viscosity was determined using a Brookfield viscometer with spindle number 2 at 30 rpm and 25°C. Foam height was measured by shaking 0.1 g of shampoo in 10 mL of water in a test tube for 20 seconds, then recording the foam height. These evaluations were conducted on days 0, 7, 14, 21, and 28 [8,9].

Antifungal activity assay

The antifungal activity of the shampoo was tested using *Pityrosporum ovale* cultured on Sabouraud dextrose agar (SDA) medium (Merck). Cultures were prepared using the slant agar method under laminar airflow (Esco). A loop of 2-day-old yeast was streaked onto agar, covered with sterile cotton wool, and incubated for 48 hours at 37°C for subsequent antifungal testing [8-11].

Five paper discs (5 mm in diameter) (OXOID, UK) were placed on each petri dish containing SDA medium. The discs were treated with 10 µL of the following formulations: NC (negative control, no ethanol extract), F1 (2% ethanol extract), F2 (3% ethanol extract), F3 (4% ethanol extract), and a positive control (1% commercial brand X shampoo). The plates were incubated for five days at 37°C, and the diameter of the inhibition zone (clear zone) was measured using a Vernier caliper. Each treatment was replicated three times [8-11].

Data analysis

Organoleptic data were analyzed descriptively, while pH, viscosity, and foam height were statistically analyzed using one-way ANOVA if the data met normality assumptions; otherwise, the Friedman Test was applied. Inhibition zone diameters were analyzed with one-way ANOVA, and non-normal data were assessed using the Kruskal-Wallis Test.

Results

Extraction results and phytochemical screening

The weight of the extract obtained from 200 grams of simplicia powder which was macerated using 70% ethanol was 33.15 grams, then a yield calculation was carried out with the aim of showing the number of components that could be extracted from a sample by the solvent and an extract yield of 16.57% was obtained.

Phytochemical screening in this study aims to identify groups of compounds in the ethanol extract of water spinach using a color reagent [12]. The ethanol extract of water spinach in this study contains polyphenolic compounds, flavonoids and quinones, the complete details are shown in Table 2.

Evaluation of physical properties of shampoo

Shampoos containing different concentrations of water spinach extract were evaluated for their physical properties, including organoleptic characteristics, pH, viscosity, and foam height (Figure 1).

The three shampoo formulations (F1, F2, F3) all exhibited a thick liquid consistency, brown color, and a characteristic water spinach odor (Table 3). The intensity of the brown color varied among the formulations, with higher extract concentrations resulting in a deeper brown. Similarly, the odor became more pungent with increasing extract concentration. Importantly, these organoleptic properties remained stable over 28 days of storage, indicating that the formulations did not undergo noticeable changes in appearance, color, or odor.

The addition of water spinach extract did not significantly affect the pH of the shampoo formulations ($p>0.05$) (Figure 1A). All formulations maintained a pH within the acceptable range established by Indonesian National Standard (SNI No. 06-2692-1992), which is 5.0-9.0. The pH values for the three formulations ranged from 5.0 to 7.5, demonstrating compliance with these guidelines [13].

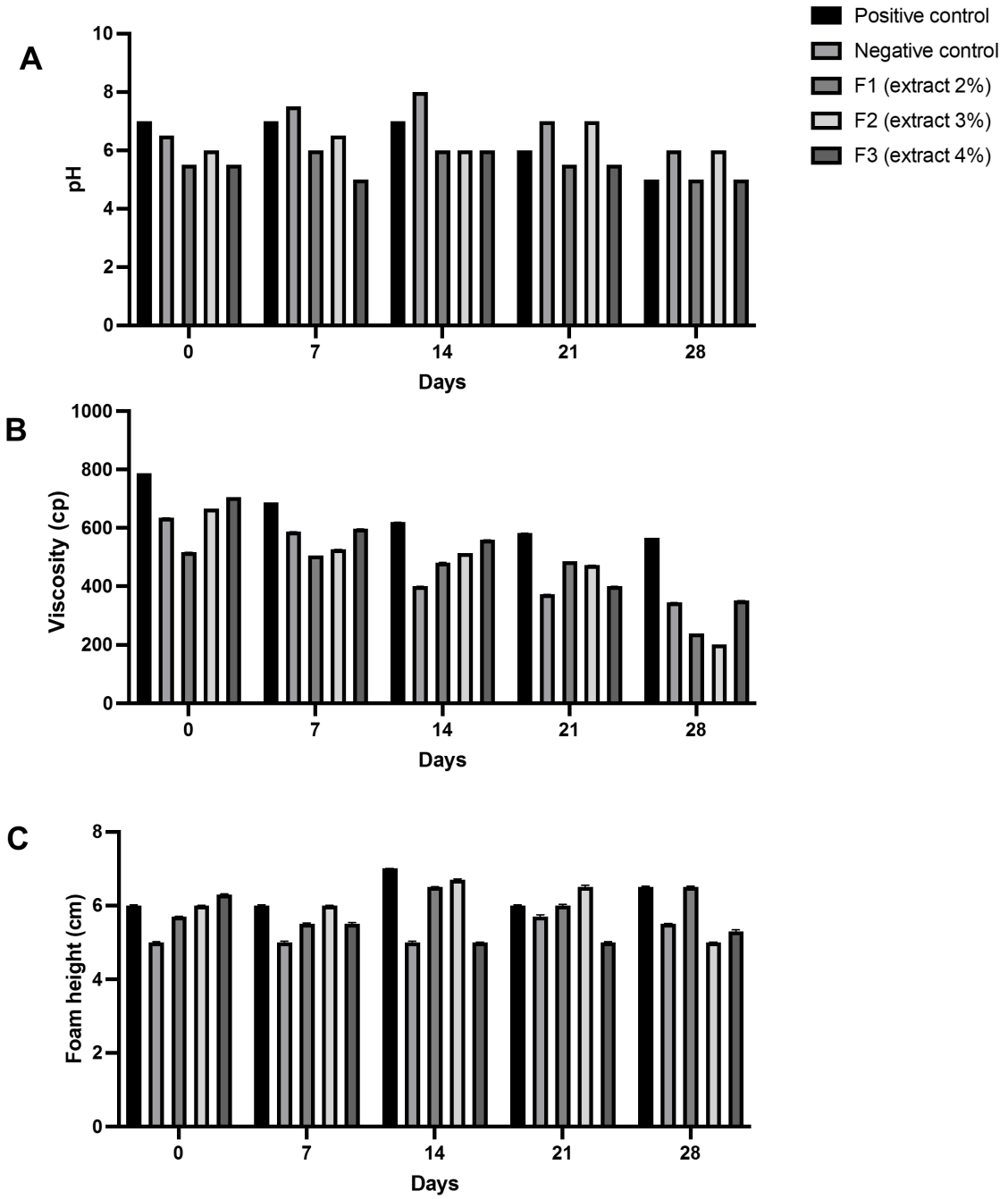

Figure 1. Evaluation of physical properties of shampoo during 28 days. (A) pH, (B) viscosity, (C) foam height

The concentration of water spinach extract significantly influenced the viscosity of the shampoo ($p<0.01$) (Figure 1B). During the first three weeks of storage, all formulations maintained viscosities within the standard range for liquid shampoos, which is 400-4000 centipoise (cp) [14]. However, by the 28th day of storage, the viscosity of all formulations, including the negative control, had fallen below the minimum standard value for shampoo preparations (<400 cps).

The foam height of the three formulations ranged from 5.0 to 6.7 cm, meeting the standard foam height for shampoos, which is between 1.3 and 22 cm [16]. The addition of water spinach extract did not significantly affect the foam height ($p>0.05$) (Figure 1C).

Antifungal activity assay

The antifungal activity of the shampoo formulations, each containing varying concentrations of water

Table 3. Evaluation of physical properties of shampoo during 28 days

Evaluation	Days to	Groups				
		NC	PC	F1	F2	F3
Form	0	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	7	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	14	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	21	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	28	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
Color	0	Clear	Clear	Greenish brown	Brown	Dark brown
	7	Clear	Clear	Greenish brown	Brown	Dark brown
	14	Clear	Clear	Greenish brown	Brown	Dark brown
	21	Clear	Clear	Greenish brown	Brown	Dark brown
	28	Clear	Clear	Greenish brown	Brown	Dark brown
Smell	0	Menthol	Spesific	Spesific	Spesific	Spesific
	7	Menthol	Spesific	Spesific	Spesific	Spesific
	14	Menthol	Spesific	Spesific	Spesific	Spesific
	21	Menthol	Spesific	Spesific	Spesific	Spesific
	28	Menthol	Spesific	Spesific	Spesific	Spesific

NC: negative control, PC: positive control

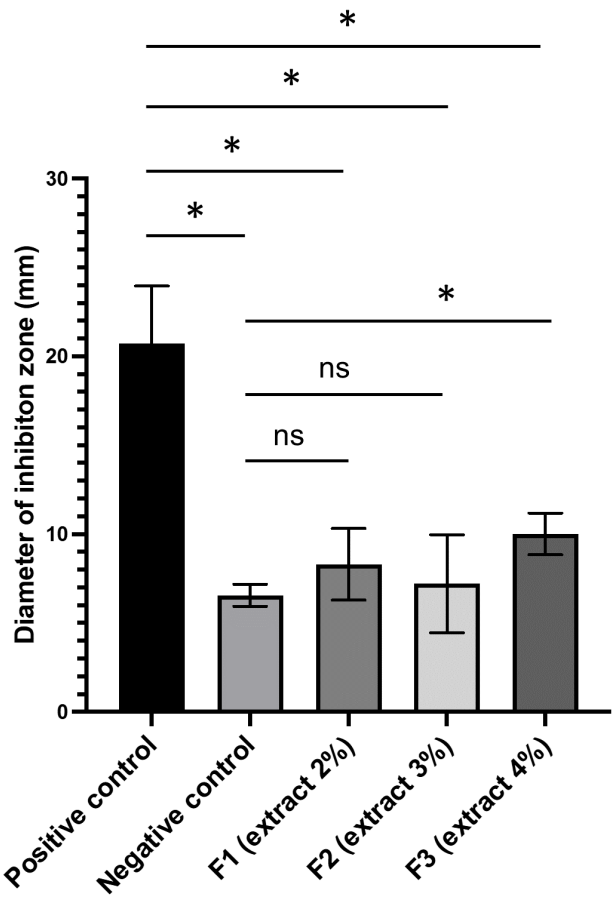

Figure 2. Diameter of inhibition zone for antidandruff shampoo. *p<0.05, ns: not significant

spinach extract, was tested against *Pityrosporum ovale*. The largest inhibition zone was observed in the F3 formulation (4% extract), with a diameter of 10.01 mm, while the smallest inhibition zone occurred in F2 (3% extract), at 7.21 mm (Figure 2). Interestingly, F1 (2% extract) produced a larger inhibition zone (8.3 mm) than F2. These results suggest that higher concentrations of water spinach extract generally increase antifungal activity, although F2 showed an unexpected result.

Statistical analysis revealed that the antifungal activity of the three formulations differed significantly from the positive control ($p<0.05$). The shampoo containing 4% water spinach ethanol extract (F3) demonstrated higher antifungal activity compared to F2 (3%) and F1 (2%). Importantly, F3 showed a significant difference in antifungal activity compared to the negative control ($p<0.05$).

Discussion

This study formulated a shampoo using ethanol extract from water spinach as the active ingredient. Phytochemical analysis confirmed that the water spinach extract contains polyphenolic compounds, flavonoids, and quinones, aligning with previous research that has reported similar findings in water spinach extracts [4,6]. In addition, the extract contains

alkaloids, tannins, triterpenoids, and saponins, all of which have recognized antimicrobial properties [4,6]. Based on these findings, the formulation of an anti-dandruff shampoo using water spinach ethanol extract was pursued.

The pH analysis of the three shampoo formulations showed compliance with the Indonesian National Standard (SNI No.06-2692-1992), which requires a pH range between 5.0 and 9.0 for shampoo products [13]. Maintaining an appropriate pH is crucial, as shampoos outside this range can irritate the scalp [7]. The observed pH values of 5.0–7.5 across the formulations confirm their suitability for use on the scalp.

However, the viscosity of all shampoo formulations, including the negative control, fell below the standard value for shampoo preparations (<400 cps) after 28 days of storage. This decrease in viscosity can be attributed to the use of Na CMC, a hydrophilic compound that absorbs water to enhance viscosity. During storage, Na CMC can undergo oxidation due to exposure to air, leading to the breakdown of its colloidal structure and a subsequent drop in viscosity [15]. This indicates the need for further formulation optimization to improve the long-term stability of viscosity.

The foam height test demonstrated that all shampoo formulations met the standard range for foam height, which is important for ensuring that the shampoo remains on the hair during washing, preventing tangles. The foam heights ranged from 5.0 to 6.7 cm, meeting the standard value set by previous research [16].

Regarding antifungal activity, F1 and F2 formulations did not significantly differ from the negative control ($p>0.05$), suggesting that an ingredient in the base formula, such as methylparaben, may be contributing to the antifungal activity. Methylparaben is commonly used as a preservative in cosmetic products and can inhibit the growth of bacteria and fungi [17]. The differences in inhibitory effects across the formulations may be influenced by factors such as fungal growth sensitivity, environmental conditions, and the interaction between active ingredients and the incubation medium [18].

The antifungal activity tests revealed that the shampoo formulations containing water spinach ethanol extract fall within the “moderate” inhibition category based on standard antimicrobial classifications [7,19,20]. The antifungal activity is likely due to the presence of polyphenolic compounds, flavonoids, and quinones in the water spinach extract, which can inhibit the growth of *P. ovale*. Flavonoids, for example, are known to disrupt microbial cell walls due to differences in polarity between their alcohol groups and the lipids that make up microbial cell walls, leading to cell wall damage and eventual cell death [21].

Conclusion

Water spinach ethanol extract can be successfully formulated into a shampoo that meets the organoleptic, pH, foam height, and viscosity requirements, although viscosity did not meet the required standards during long-term storage. The shampoo demonstrated antifungal activity against *P. ovale*, with the 4% extract formulation (F3) exhibiting the highest antifungal activity compared to F2 (3%) and F1 (2%), and was significantly more effective than the negative control ($p<0.05$).

Conflict of interest

No potential conflicts of interest were reported by the authors.

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Author contributions

EG contributed to the study design, data analysis, and manuscript drafting. AB contributed to data analysis. LA contributed to data collection and manuscript drafting alongside EG. All authors reviewed and approved the final version of the manuscript.

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References

1. Basarang, M., Rianto, M.R., Julianto, R.D., Kemampuan Senyawa Anti Ketombe pada Sampo dalam Menghambat Pertumbuhan Jamur yang diisolasi dari Ketomber. . Journal of Health Science and Technology. 2022; 3(2): 134-142. https://doi.org/10.53861/lontarariset.v3i2.320
2. Kasturi Y, Rahmiati R, Rosalina L. Pengaruh Pemanfaatan Shampo Ekstrak Gambir Terhadap Perawatan Kulit Kepala Berketombe. Journal Of Home Economics and Tourism. 2018 Jul 20;14(1).
3. Rook A, Dawber R. 1991. Diseases of The Hair and Scalp.

4. Baura Va, Pareta Dn, Tulandi Ss, Untu Sd. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kangkung Air Ipomoea Aquatica Forsk Terhadap Bakteri Staphylococcus Aureus. Biofarmasetikal Tropis (The Tropical Journal Of Biopharmaceutical). 2021 Apr 28;4(1):10-20. https://doi.org/10.55724/j.biofar.trop.v4i1.303
5. Puspita. Perbandingan Efektivitas Ekstrak Daun Kangkung (Ipomea Reptans) Dengan Ketokonazol 1% Secara In Vitro Terhadap Pertumbuhan Pityrosporum ovale Pada Ketombe - Core. 2020.
6. Yuliana A. Aktivitas Kangkung Air (Ipomoea aquatica) Terhadap Jamur Pityrosporum ovale Hasil Isolasi Secara In Vitro. Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-Ilmu Keperawatan, Analis Kesehatan Dan Farmasi. 2015 Jun 10;9(1):1. https://doi.org/10.36465/jkbth.v9i1.87
7. Malonda Tc. Formulasi Sediaan Sampo Antiketombe Ekstrak Daun Pacar Air (Impatiens Balsamina L.) Dan Uji Aktivitasnya Terhadap Jamur Candida Albicans ATCC 10231 Secara In Vitro. Pharmacon. 2017;6(4).
8. Arianto, A., Sitorus, P., Ma'rufah, R. Formulasi dan Evaluasi Aktivitas Antijamur Gel Sampo Anti Ketombe Minyak Sereh Dapur (Cymbogopon citratus). Tropical Medicine. 2018;1(3): 7-13p. https://doi.org/10.32734/tm.v1i3.253
9. Genatrika, E., Nurkhikmah, I., Hapsari, I., Formulasi Sediaan Krim Minyak Jintan Hitam (Nigella sativa L.) sebagai Antijerawat Terhadap Bakteri Propionibacterium acnes. Jurnal PHARMACY. 2016; 13(2):192-201.
10. Sitompul Mb. Formulasi Dan Uji Aktivitas Sediaan Sampo Antiketombe Ekstrak Etanol Daun Alamanda (Allamanda Cathartica L.) Terhadap Pertumbuhan Jamur Candida Albicans Secara In Vitro. Pharmacon. 2016;5(3).
11. Suryani, A.I., Rohwah, E.I., Uji Aktivitas Sediaan Sampo Antiketombe Ektrak Daun ketepeng cina (Cassia alata L.) terhadap jamur Pityrosporum ovale.2023;5 (3): 411-423. https://doi.org/10.37311/jsscr.v5i3.24310
12. Haryanto, Ariawan M.W., Utami Y.P., Duppa, M.T., Ghozaly M.R., Paerah, I.A.P., Junita, F., Salman, Puspitasari, A., Rahmadani, E.H. Fitokimia dan Farmakologi. 1st Ed. Purbalingga: Eureka Media Aksara; 2024
13. Komite Sni. Sni 8860:2020 Sampo Cair. Badan Standarisasi Nasional; 2020.
14. Shcmitt Wh, Williams Df, Routledge H, Routledge C. Chemistry and The Technology of The Cosmetics and Toiletries Industry. J Chem Educ. 1993 Jan 1;70(1):A25. https://doi.org/10.1021/ed070pA25.8
15. Rahmaningtyas E, Yusa Nm, Ni Nyoman Nn. Pengaruh Penambahan CMC (Carboxyl Methyl Cellulose) Terhadap Karakteristik Sirup Salak Bali (Salacca Zalacca Var. Amboinensis) Selama Penyimpanan. Jurnal Ilmu Dan Teknologi Pangan (Itepa). 2016 Dec;5(2)
16. Harry Rg. Harry's Cosmeticology. 9th Ed. Harlow : Chemical Publishing Company; 2015.
17. Sheskey P.J., Hancock, B.C., Moss, G.P., Goldfarb, D.J. 2020. Handbook of Pharmaceutical Excipients.
18. Hidayah, N., Hisan, A.K., Solikin, A. Uji Aktivitas Ekstrak Sargassum muticum sebagai Alternatif Obat Bisul Akibat Aktivitas Staphylococcus aureus. Journal of Creativity Students. 2016; 1(1):1-9. https://doi.org/10.15294/jcs.v1i2.7794
19. Susanto D, Ruga R, Sudrajat. 2013. Studi Kandungan Bahan Aktif Tumbuhan Meranti Merah (Shorea Leprosula Miq) Sebagai Sumber Senyawa Antibakteri. Lembaga Penelitian Universitas Mulawarman.
20. Kherid, M.T., Dianasari, D., Nuri, Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kacapiring (Gardenia augusta Merr.) dan Fraksinya terhadap Salmonella thypi. Pharmaceutical Journal of Indonesia. 2020; 5(2):97-102. https://doi.org/10.21776/ub.pji.2020.005.02.5
21. Jannata Rh, Gunadi A, Ermawati T. Daya Antibakteri Ekstrak Kulit Apel Manalagi (Malus Sylvestris Mill.) Terhadap Pertumbuhan Streptococcus Mutans. Jurnal Pustaka Kesehatan. 2014 Jan;2(1):23-8.