Formulation of cashew leaf extract peel-off gel mask (Anacardium occidentale L.) and its activity test against Staphylococcus epidermidis

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Abstract. Cashew leaf (*Anacardium occidentale* L.) is rich in bioactive compounds such as flavonoids, tannins, glycosides, and terpenoids, which exhibit antibacterial properties. To optimize its application, the leaf extract was formulated into a peel-off gel mask. This study aimed to evaluate both the antibacterial activity of the cashew leaf extract peel-off gel mask against *Staphylococcus epidermidis* and its physical stability. The extract was obtained through maceration using 96% ethanol as a solvent, and peel-off gel masks were prepared with extract concentrations ranging from 10% to 20%. The antibacterial efficacy of the formulations was assessed using the agar-well diffusion method, while the physical stability of the masks was monitored over a 4-week storage period. Physical evaluations included organoleptic properties, adhesiveness, spreadability, pH, and drying time. Data were analyzed using one-way ANOVA and paired T-tests. Results indicated that higher concentrations of cashew leaf extract corresponded with increased antibacterial activity, adhesiveness, and drying time, while spreadability decreased, and pH became more acidic. The peel-off gel masks demonstrated significant antibacterial activity against *Staphylococcus epidermidis*, with inhibition zone diameters of 11.25±0.50 mm for Formula 1, 12.50±0.58 mm for Formula 2, and 14.00±0.82 mm for Formula 3. Overall, the peel-off gel masks remained physically stable throughout the 4-week storage period.

1 Introduction

Microorganisms, including bacteria, viruses, and fungi, are among the most prevalent causes of skin diseases [1]. Acne, a particularly common skin condition during adolescence, often persists into adulthood. Its onset is influenced by several factors, with excessive sebum production, bacterial proliferation, and inflammation being the primary contributors [2]. Bacteria such as Propionibacterium acnes, *Staphylococcus epidermidis*, and Staphylococcus aureus are key agents in the inflammatory process associated with acne [3]. Most available anti-acne treatments incorporate synthetic antibiotics, such as erythromycin and clindamycin [4]. However, the continuous and prolonged use of such antibiotics raises concerns about bacterial resistance [5]. Furthermore, these synthetic agents are often associated with adverse side effects, including dry skin, rashes, and pruritus [6]. Given these concerns, it is imperative to explore alternative treatments for acne that offer comparable or superior efficacy to synthetic antibiotics. One promising approach lies in the use of plant-based therapies [7]. Natural ingredients are perceived as a safer alternative, with a reduced risk of side effects [8][9]. Cashew leaves (*Anacardium occidentale* L.) represent a potential natural remedy for acne, as they are rich in bioactive compounds such as flavonoids, tannins, glycosides, and terpenoids [10]. These compounds contribute to the leaves' antibacterial and antioxidant properties [11]. Previous studies have demonstrated the antibacterial efficacy of cashew leaf extract against a range of bacterial species, including *Staphylococcus aureus* (20 mm inhibition zone), *Bacillus subtilis* (19 mm), *Pseudomonas aeruginosa* (13 mm), and *Escherichia coli* (11 mm) [12]. Moreover, research by Ayu et al. (2013) revealed that a 15% concentration of cashew leaf extract produced an inhibition zone diameter of 11 mm against *Staphylococcus epidermidis* [13]. In this study, cashew leaf extract was formulated into a peel-off gel mask to enhance its application. Peel-off gel masks are widely used in treating facial skin conditions such as acne, wrinkles, and premature aging. These masks, upon drying, form a thin, uniform film that can be easily peeled off. Typically, they dry within 5 to 30 minutes and provide a moisturizing effect to the skin [14].

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2 Materials and Method

2.1 Materials

Cashew leaves (*Anacardium occidentale* L.) were sourced from Simo, Boyolali, Central Java, Indonesia. *Staphylococcus epidermidis* bacteria were obtained from the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia. The additional materials used in the study included 96% ethanol (Bratachem), Dimethyl Sulfoxide (DMSO) (Merck), 0.9% NaCl (Bratachem), distilled water, Mueller Hinton (MH) medium (Oxoid), Brain Heart Infusion (BHI) medium (Himedia), Mannitol Salt Agar medium (Oxoid), erythromycin gel (Erymed®), Polyvinyl alcohol (PVA) (Merck), Propylene glycol, Polyvinyl pyrrolidone (PVP) (Merck), Sodium lauryl sulfate (Merck), methyl paraben, propyl paraben, sodium metabisulfite (Merck), and Sodium Ethylene Diamine Tetraacetate (Sod EDTA) (E-Merck).

2.2 Extraction

The cashew leaves were thoroughly cleaned and subsequently dried in an oven at 60 °C for 24 hours until completely dehydrated. The dried leaves were then ground into a fine powder using a blender to reduce particle size and increase surface area. A total of 650 grams of the powdered cashew leaves were soaked in 4.9 liters of 96% ethanol in a sealed vessel, ensuring protection from light. The mixture was left to macerate for five days, during which it was stirred regularly. After the five-day maceration process, the resulting extract was filtered through a Buchner funnel, followed by evaporation using a rotary evaporator. The remaining extract was further concentrated in a water bath at 60 °C until a thick, viscous extract was obtained.

2.3 Formulation of Cashew Leaf Extract Peel-off Gel Mask

The peel-off gel mask containing cashew leaf extract was prepared in four different formulations, each with varying concentrations of the extract (Table 1).

Table 1. Composition of Cashew Leaf Extract Peel-off Gel Mask

Materials	Formula (% b/b)			
	F0	**F1**	**F2**	**F3**
Extract of Cashew leaves	-	10	15	20
PVA	10	10	10	10
Propylene glycol	15	15	15	15
PVP	3	3	3	3
Sod. lauryl sulfate	2	2	2	2
Methyl paraben	0.15	0.15	0.15	0.15
Propyl paraben	0.05	0.05	0.05	0.05
Sodium Metabisulfit	0.1	0.1	0.1	0.1
Sodium EDTA	0.1	0.1	0.1	0.1
Ethanol 96%	20	20	20	20
Aquadest till	100	100	100	100

To prepare the peel-off gel mask, polyvinyl alcohol (PVA) was first hydrated with water, followed by heating the mixture to 80 °C under continuous stirring until the PVA was fully swollen, yielding a transparent solution. The mixture was then cooled. In parallel, polyvinyl pyrrolidone (PVP) was dissolved in 96% ethanol. Sodium metabisulfite was dissolved in a small amount of distilled water, then added to the cashew leaf extract. Methyl paraben and propyl paraben were dissolved in propylene glycol, while sodium lauryl sulfate and sodium EDTA were dissolved in distilled water. Finally, the swollen PVA was combined with all the ingredients and homogenized [15]. Notably, PVP exhibits swelling properties, making it suitable for use as a binding agent in gel formulations and tablets [16].

2.4 Stability testing of cashew leaf extract peel-off gel mask

The stability of the cashew leaf extract peel-off gel mask formulations was assessed over a four-week period, with evaluations conducted weekly. The parameters analyzed included organoleptic properties, spreadability, adhesiveness, pH, and drying time.

2.5 Antibacterial test

The antibacterial efficacy of the formulations was evaluated using the well diffusion method. The bacterial suspensions were adjusted to a turbidity equivalent to the McFarland standard (1.0 × 10^8 CFU/mL) by adding 0.9% NaCl. The suspension was spread onto petri dishes containing solidified Mueller Hinton (MH) medium using a glass spreader. The agar surface was divided into five sections, and wells were created using a No. 3 cork borer. Each well was filled with 50 µL of the following samples: 10%, 15%, or 20% gel containing cashew leaf extract, dimethyl sulfoxide (negative control), and erythromycin gel (positive control). The plates were incubated at 37 °C for 24 hours, and the inhibition zones were measured using a ruler.

2.6 Data analysis

The experimental data were analyzed using SPSS 23.0 software to assess differences in inhibition zone diameters between cashew leaf extract and the peel-off gel mask formulations at various extract concentrations, using a one-way ANOVA. The physical characteristics of the gel masks, including adhesiveness, spreadability, pH, and drying time, were evaluated in both the first and fourth weeks, with a one-way ANOVA employed to determine significant differences between the

formulations. Additionally, a paired T-test was conducted to assess the stability of the cashew leaf extract peel-off gel mask over the four-week storage period.

3 Results and discussion

In this study, the extraction of cashew leaves was performed using the maceration method with 96% ethanol as the solvent, selected for its ability to effectively extract compounds such as flavonoids, phenols, alkaloids, sterols, tannins, and terpenoids [13]. The maceration process yielded 14.16% of extract. Prior to incorporating the extract into a peel-off gel mask formulation, an antibacterial activity assay was conducted to verify the extract's efficacy against *Staphylococcus epidermidis*. This preliminary assessment utilized the well diffusion method, which has been shown to produce larger inhibition zones compared to the disk diffusion method, as noted in Prayoga's (2013) research [17].

Dimethyl sulfoxide (DMSO) was employed as the negative control, while a 2% erythromycin gel served as the positive control in the extract assay. Upon confirming the antibacterial activity of the cashew leaf extract in this preliminary test, the extract was subsequently formulated into a peel-off gel mask. Peel-off gel masks form a film that dries within 5 to 30 minutes and can be easily peeled off after application [14]. For the antibacterial activity assay of the formulated mask, Formula 0 (F0), which did not contain the extract, was used as a negative control, while the 2% erythromycin gel was retained as the positive control. Erythromycin, a narrow-spectrum antibiotic, exhibits greater efficacy against Gram-positive bacteria and is commonly prescribed for skin and soft tissue infections [18].

The results of the antibacterial activity tests, conducted in four replicates, indicated that a 20% concentration of cashew leaf extract exhibited the highest inhibition zone, measuring 11.50 mm. When formulated into a peel-off gel mask, this concentration produced an inhibition zone with a diameter of 14.00 mm (Table 2). These findings are consistent with previous research, which demonstrated an inhibition zone of 11 mm against *Staphylococcus epidermidis* at a 15% concentration of cashew leaf extract [13].

Table 2. Antibacterial activity of cashew leaf extract and cashew leaf extract peel-off gel mask against *Staphylococcus epidermidis* bacteria (n=4)

Extract concentration (50 µL/well)	**inhibition (mm)**	**peel-off gel mask (100 mg/well)**	**inhibition (mm)**
Extract 10%	9.50 ± 0.58	F1 (10%)	11.25 ± 0.50
Extract 10% 15%	9.75 ± 0.58	F2 (15%)	12.50 ± 0.58
Extract 10% 20%	11.50 ± 1.41	F3 (20%)	14.00 ± 0.82
DMSO (-)	7.00 ± 0	F0 (0 %)	9.00 ± 0*
Erytromycin gel (+)	18.75 ± 0.73		

*= irradical

The one-way ANOVA analysis of the antibacterial activity test for the cashew leaf extract yielded a p-value of less than 0.05, indicating a statistically significant difference in inhibition zone diameter across the different concentrations of the extract. Similarly, the one-way ANOVA analysis for the antibacterial activity of the cashew leaf extract peel-off gel mask also resulted in a p-value of <0.05, confirming a significant difference in inhibition zone diameter among the various concentrations of cashew leaf extract in the peel-off gel mask.

A paired T-test comparison between the 20% extract and Formula 1 (10% extract) revealed a p-value greater than 0.05, indicating that incorporating the cashew leaf extract into a peel-off gel mask did not alter its antibacterial efficacy. The results also demonstrated that as the concentration of the extract increased, so did the diameter of the inhibition zone. Formula 3 (F3), which contained 20% cashew leaf extract, exhibited the highest inhibition zone diameter.

The organoleptic evaluation, aimed at assessing changes in color, odor, and homogeneity of the cashew leaf extract peel-off gel mask during storage, revealed no observable changes over the 4-week storage period.

The stickiness test was conducted to assess the adhesive properties of the peel-off gel mask upon application to the skin [19]. One-way ANOVA testing for adhesive power across both the 1st and 4th weeks yielded a p-value of less than 0.05, suggesting a significant difference in adhesiveness between the different formulations. The concentration of cashew leaf extract was found to have a direct influence on adhesive strength, with higher concentrations leading to greater adhesive power. Specifically, Formula 3 (F3), containing 20% cashew leaf extract, exhibited the longest adhesion time on the skin (Fig. 1). A paired T-test analysis further indicated that all formulas maintained stable adhesive properties throughout the 4-week storage period, as the p-value exceeded 0.05 in both the 1st and 4th weeks.

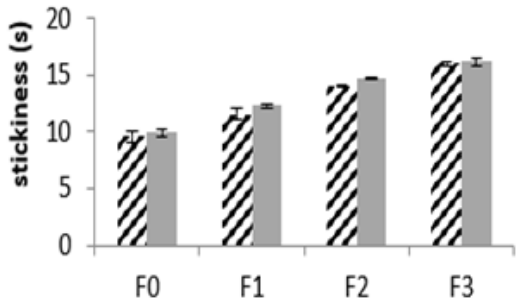

Fig 1. Adhesive strength of cashew leaf extract peel-off gel mask during the 1st and 4th weeks

The spreadability test evaluates how well the peel-off gel mask spreads when applied to the skin, with an ideal mask easily distributing over the surface.

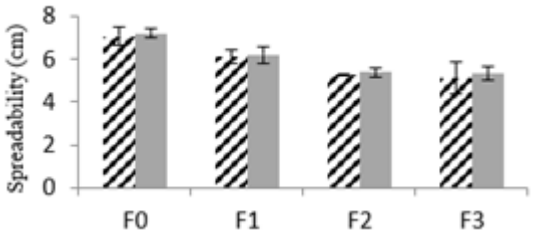

Fig 2. The spreadability of cashew leaf extract peel-off gel mask in the first and fourth weeks

A one-way ANOVA test conducted in both the 1st and 4th weeks yielded a p-value of less than 0.05, indicating a statistically significant difference in the spreadability of the various formulations over time. The spreadability of the peel-off gel mask is inversely related to the concentration of cashew leaf extract. Higher concentrations of extract reduce the mask's ability to spread, with Formula 3 (F3), containing the highest concentration of 20% extract, demonstrating the least spreadability compared to the other formulations (Fig. 2). According to paired T-test analysis, the p-value was greater than 0.05 for all formulas during both the 1st and 4th weeks, indicating that the spreadability of the cashew leaf extract peel-off gel masks remained stable throughout the 4-week storage period.

The pH test was conducted to determine the acidity level of the mask, which is crucial for ensuring its safety when applied to the skin. The optimal pH range for skincare products is between 4 and 8, as excessively acidic formulations can cause irritation, while highly alkaline products can lead to dryness and scaling of the skin [20]. Test results indicated that all formulas fell within this safe pH range, with values between 4.67 and 5.42. Formula 3 (F3), which contains the highest concentration of extract, exhibited a more acidic pH due to the naturally low pH of cashew leaf extract (pH 3.13) (Fig. 3).

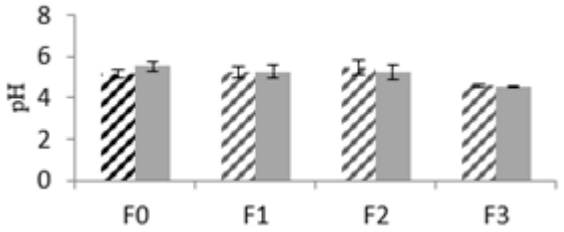

Fig 3. pH of cashew leaf extract peel-off gel mask during the first and fourth weeks

A one-way ANOVA analysis on pH levels in weeks 1 and 4 showed a p-value of less than 0.05, signifying a significant difference in pH between the formulations over time. However, paired T-test analysis yielded a p-value greater than 0.05 for all formulas, indicating that the pH of the cashew leaf extract peel-off gel mask remained stable over the 4-week storage period.

The drying time test assesses the length of time required for the mask to dry completely after application to the skin [21-24]. Typically, peel-off gel masks dry within 5 to 30 minutes [14]. Results from the drying time test revealed that all formulations dried in under 30 minutes (Fig. 4). Formula 0 (F0) required the longest drying time due to its higher water content, while Formula 3 (F3), which has a lower water content, dried the fastest.

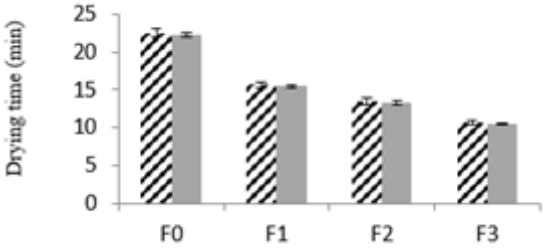

Fig 4. Drying time of cashew leaf extract peel-off gel mask during the first and fourth weeks

According to one-way ANOVA analysis conducted in weeks 1 and 4, a p-value of less than 0.05 was obtained, indicating a significant difference in drying times among the various formulations. However, paired T-test analysis showed a p-value greater than 0.05 for all formulas in both weeks 1 and 4, signifying that the drying time of the cashew leaf extract peel-off gel masks remained consistent and stable over the 4-week storage period.

4 Conclusions

This study concludes that increasing the concentration of cashew leaf extract in the peel-off gel mask formulation significantly enhances its adhesiveness and extends its drying time, while reducing its spreadability and increasing its acidity. The peel-off gel mask, incorporating cashew leaf extract, demonstrated remarkable stability over a four-week storage period and exhibited strong antibacterial activity against *Staphylococcus epidermidis*. Additionally, higher concentrations of cashew leaf extract in the formulation correlated with a larger inhibition zone.

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6 Authors contributions

Setyo Nurwaini contributed to the overall study design and authored the manuscript. Intan Putri Hakiki supervised the laboratory work. Ika Trisharyanti Dian Kusumowati contributed to the collection and analysis of antibacterial data.

7 Conflict of interest

The authors declare no conflict of interest

References

1. J. Paul, Introduction to infectious diseases, in *Disease Causing Microbes*, ed. J. Paul (Cham: Springer International Publishing, 20240
2. G.F. Webster, Acne vulgaris, (Department of Dermatology Jefferson Medical College, 325, 475–479, 2002)
3. I.B.S Sitohang, H. Fathan, E. Effendi, and M.Wahid, The susceptibility of pathogens associated with acne vulgaris to antibiotics. Medical Journal of Indonesia, **28** (1), 21–27 (2019). https://doi.org/10.13181/mji.v28i1.2735
4. S. Feldman, R.E. Careccia, K.L. Barham, and J. Hancox, Diagnosis and Treatment of Acne. American Family Physician, **69** (9), 2123–2130 (2004).
5. W. Madelina and S.Sulistiyaningsih, Review: Antibiotic Resistance In Acne Treatment Therapy. Farmaka, **16** (2), 105–117 (2018). https://doi.org/10.24198/jf.v16i2.17665
6. S. Agnihotri , S.Wakode, and A. Agnihotri, Formulation And Evaluation of Herbal Antiacne of Myrica Esculenta. Asian Journal of Pharmaceutical and Clinical Research, **9** (4), 358–361 (2016). https://journals.innovareacademics.in/index.php/ajpcr/article/view/12385
7. Y.R.D. Ratna, U.S. Ardani, Z. Fathiana, A. Rahmatillah, and I.T.D. Kusumowati, Antibacterial Power of Cashew Leaf Extract and Fractions (*Anacardium occidentale* L.) Against Sensitive and Multiresistant *Staphylococcus aureus* Bacteria. Jurnal Ilmu Kefarmasian Indonesia, **14** (1), 103–110 (2017).
8. M.S. Qomar, M.A.K. Budiyanto, S. Sukarsono, S. Wahyuni, and H. Husamah, Effectiveness of Various Concentrations of Cinnamon Leaf Extract (*Cinnamomum burmannii* [Ness.] BI) on the Diameter of the Growth Inhibition Zone of *Staphylococcus epidermidis* Bacteria. Jurnal Biota, **4** (1), 12–18 (2018). https://doi.org/10.19109/Biota.v4i1.1454
9. S. Laianto, R. Sari, and L. Pratiwi, Effectivity Test Of Anti-Acne Gel Preparation of Ethanol Ekstract From Bitter Melon Fruit (*Momordica charantia*) against *Staphylococcus epidermidis* and *Propionibacterium acnes* by Diffusion Method. Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN, 1(1), (2014)
10. A.A. Mustapha, G. Owuna, J.O. Ogaji, U.I. Is, and M.M Idris M.M., Phytochemical Screening and Inhibitory Activities of *Anacardium occidentale* Leave Extracts against Some Clinically Important Bacterial Isolates. International Journal of Pharmacognosy and Phytochemical Research, **7** (2), 365–369 (2015).
11. O.O. Ajileye, E.M. Obuotor, E.O. Akinkunmi, and M.A. Aderogba, Isolation and characterization of antioxidant and antimicrobial compounds from *Anacardium occidentale* L. (Anacardiaceae) leaf extract. Journal of King Saud University-Science, **27** (3), 244–252 (2015). http://dx.doi.org/10.1016/j.jksus.2014.12.004.
12. A.P. Dahake, V.D. Joshi, and A.B. Joshi, Antimicrobial Screening of Different Extract of *Anacardium occidentale* Linn. Leaves. International Journal ChemTech Research, **1** (4), 856–858 (2009)
13. D. Ayu, P. Indrayudha, and I.T.D Kusumowati, Aktivitas antibakteri kombinasi ekstrak etanol daun jambu monyet (*Anacardium occidentale* L.) Dan Vankomisin Terhadap *Staphylococcus aureus* Dan *Staphylococcus epidermidis*. Skripsi, Fakultas Farmasi, Universitas Muhammadiyah Surakarta, Surakarta (2013)
14. C. Darsika, K.V. Sowmya, K. Suganya, X.F.Grace, and S. Shanmuganathan, Preparation and Evaluation of Herbal Peel Off Face Mask. American Journal of Pharmtech Research, **5** (4), 332-336 (2015)
15. Y. Noviani, S.U. Noor, and E. Nengsih, The Effect of Variation in Polyvinyl Alcohol (PVA) Concentration on Peel-Off Gel Mask Formulation of Starfruit Extract (*Averrhoa bilimbi* L.) as an Anti-Acne. Jurnal Ilmu Kefarmasian Indonesia, **14** (2), 199–205 (2016)
16. G.S. Alita and S. Suprapto, Optimization of Captopril Slow Release Tablets Using PVP as a Binder and Talc-Magnesium Stearate as a Lubricant Using The Simplex Lattice Design Method. Usadha Journal of Pharmacy, **2** (1), pp. 86–107 (2023). https://doi.org/10.23917/ujp.v2i1.121
17. E. Prayoga, Comparison Effect of Betel Leaf Extract (*Piper betle* L.) with method Diffusion Disk and Methods Hole cup towards the Growth of *Staphylococcus aureus*. Skripsi, Fakultas Kedokteran dan Ilmu Kesehatan, Universitas Islam Negeri Syarif Hidayatullah, Jakarta (2013).
18. I.R. Rahman, I.T.D. Kusumowati, P. Indrayudha, and A. Sukmawati, Physical Stability And Antibacterical Activity Oferythromycin Suspension Using Pulvis Gummi Arabici as a Suspending Agent. Pharmacon, **12** (2), 44–49 (2011). 10.23917/pharmacon.v12i2.30
19. W. Diniawati, S. Nurwaini, Formulasi masker gel peel off ekstrak daun pandan wangi (Pandanus amaryllifolius roxb.) dan aktivitasnya terhadap Staphylococcus epidermidis. Usadha Journal of Pharmacy, 2(3), 416–428 (2023). https://doi.org/10.23917/ujp.v2i3.124
20. M. Shukr and G. Metwally G., Evaluation of Topical Gel Bases Formulated with Various Essential Oils for Antibacterial Activity against Methicillin- Resistant *Staphylococcus aureus.*

Tropical Journal of Pharmaceutical Research, 12 (6), 877–884 (2013).

21. D. Rahmawanty, N. Yulianti, and M. Fitriana, Formulation And Evaluation Peel-off Facial Mask Containing Quercetin With Variation Concentration Of Gelatin And Gliserin. Media Farmasi, **12** (1), 17–32 (2015). http://dx.doi.org/10.12928/mf.v12i1.3019
22. R. Setiawati, A. Sukmawati, Physical characteristics and antioxidant activity of peel off gel face mask containing *Hylocerus polyrhizus* fruit juice. Pharmacon: Jurnal Farmasi Indonesia, **15** (2), p. 65-74 2018) 10.23917/pharmacon.v15i2.7245
23. Y.A. Dhiafanti and G. Setiyadi, Optimization of HPMC as A Gelling Agent and Glycerin as A Humectant in Gel Mask Preparation Of Chayote (Sechium edule (Jacq.) Swartz) Extract. Usadha Journal of Pharmacy, **2**(1), pp. 1–15 (2023). https://doi.org/10.23917/ujp.v2i1.93
24. G. Setiyadi and A. Qonitah, Optimization of Gel Peel-Off Mask Ethanolic Extract of Betel Leaf (*Piper betle* L.) with Carbomer and Polivinyl Alcohol Combination. Pharmacon: Jurnal Farmasi Indonesia, **17**(2), p. 174-183 (2020). 10.23917/pharmacon.v17i2.11976