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Research Article

Gel Formulation of Awar-awar Leaves Ethanol Extract and In Vitro Anti-inflammatory Activity Evaluation

Formulasi Gel Ekstrak Etanol Daun Awar-awar dan Evaluasi Aktivitas Anti-inflamasi secara In Vitro

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ABSTRACT

Gel is a semisolid dosage form that utilises a gelling agent, such as Carbopol 940, providing ease of application and efficient skin absorption. Ethanol extract of *awar-awar* (*Ficus septica* Burm. F*)* leaves, which contain flavonoids and tannins, has demonstrated promising anti-inflammatory potential. This study aimed to formulate and evaluate the stability of a gel containing the ethanol extract of *awar-awar* leaves, as well as assess its anti-inflammatory effects. The methods comprised phytochemical screening, gel formulation and evaluation, stability testing, and an in vitro anti-inflammatory assay. Four gel formulations were created: F0 (0%), F1 (1.5%), F2 (3%), and F3 (4.5%). Evaluation results showed that F0, F1, and F3 were homogeneous, with viscosities between 5,000-50,000 mPa·s, spreadability of 5-7 cm, and adhesion time exceeding 2 seconds, meeting Indonesian National Standard (SNI) requirements. However, pH testing during the cycling tests revealed instability in formulations F1 through F3 during storage. The anti-inflammatory test, conducted using the protein denaturation inhibition method, showed all three formulations had anti-inflammatory activity, with F3 being the most effective (92.20% inhibition). Therefore, F3 is considered the most promising formulation due to its high anti-inflammatory activity and acceptable pharmaceutical characteristics for gel preparations.

Keywords: Anti-inflammatory, *Awar-awar*, Gel Formulation, In Vitro, Protein Denaturation

ABSTRAK

Gel merupakan sediaan semisolid yang menggunakan bahan pembentuk gel seperti Karbopol 940. Gel memberikan kenyamanan ketika digunakan dan mudah berpenetrasi ke dalam kulit. Bahan alami seperti ekstrak etanol daun awar-awar (Ficus septica Burm. F*), memiliki kandungan flavonoid dan tanin yang sangat berpotensi sebagai antiinflamasi. Penelitian ini bertujuan untuk mengetahui formulasi dan stabilitas sediaan gel yang mengandung ekstrak etanol daun awar-awar, serta menguji aktivitas antiinflamasi. Metode penelitian ini meliputi skrining fitokimia, formulasi dan evaluasi sediaan gel, uji stabilitas sediaan, dan uji aktivitas antiinflamasi secara in vitro. Gel dibuat dalam empat formula, yaitu F0 (0%), F1 (1,5%), F2 (3%), dan F3 (4,5%). Hasil*

evaluasi sediaan menunjukkan bahwa F0, F1, dan F3 homogen, memiliki viskositas 5.000-50.000 mPa·s, daya sebar 5-7 cm, dan daya lekat >2 detik, memenuhi persyaratan Standar Nasional Indonesia (SNI). Namun, hasil uji pH saat cycling test menunjukkan F1, F2, dan F3 tidak stabil selama penyimpanan. Uji aktivitas antiinflamasi dengan metode penghambatan denaturasi protein, menunjukkan bahwa ketiga formulasi memiliki aktivitas antiinflamasi, dengan F3 sebagai formula paling efektif (inhibisi 92,20%). Oleh karena itu, F3 merupakan formula yang paling menjanjikan karena aktivitas antiinflamasi yang tinggi dan karakteristik farmasetika yang memenuhi syarat sediaan gel.

Kata Kunci: *Antiinflamasi, Awar-awar, Denaturasi Protein, Formulasi Gel, In Vitro*

INTRODUCTION

Inflammation is a biological response essential for protecting the body and accelerating tissue repair processes (1). Data from 2018 show that 19.8% of Indonesian households store Non-Steroidal Anti-inflammatory Drugs (NSAIDs), reflecting the high prevalence of inflammatory conditions in the country (2). Although oral synthetic NSAIDs are effective in managing inflammatory symptoms, their long-term use is associated with adverse effects such as peptic ulcers, kidney disorders, and anaemia, particularly with prolonged use (1). As an alternative, natural ingredient-based drugs and topical administration are preferred due to their potential to reduce side effects. Topical drug delivery systems enhance bioavailability, effectiveness, and local drug concentration by bypassing first-pass metabolism, thereby making them more targeted and safer (3).

One natural ingredient with anti-inflammatory potential is *awar-awar* leaves (*Ficus septica* Burm. F). This plant grows wild and is commonly found along roadsides, in bushes, and in forest areas (4). *Awar-awar* leaves are known to contain saponins, tannins, and flavonoids. In Indonesian traditional medicine, these leaves have been used to treat skin diseases, wounds, and inflammation. Several studies have been conducted on the anti-inflammatory effects of *awar-awar* leaves. Extract of *awar-awar* leaves is known to have epithelialisation activity in rats at a concentration of 1.5%, which is effective in healing burns and inflammatory conditions (5).

Among various topical preparations, gels are particularly favoured due to their aesthetic appeal and functional advantages. Numerous herbal plants containing bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and interquinones have been formulated into gels for their anti-inflammatory benefits, for example, extracts from celery leaves, moringa leaves, torch ginger (*kecombrang*) leaves, pearl grass leaves, and *ketepeng cina* leaves. Gels offer several advantages, such as a cooling effect on the skin, provide moisturisation, and readily penetrate the skin. In addition, gels are water-soluble, making them comfortable and easy to use on the skin (6). Gel-based treatment is preferred because gels easily penetrate the skin and provide better absorption (7).

Although *awar-awar* leaves extract has shown promising anti-inflammatory potential, its formulation into a gel dosage form has rarely been investigated. In response to these considerations, this study aims to develop gel formulations of ethanol extract of *awar-awar* leaves at varying concentrations of 0% (F1), 1.5% (F2), 3% (F3), and 4.5% (F4), and to evaluate their in vitro anti-inflammatory activity using the Bovine Serum Albumin (BSA) protein denaturation inhibition method, analysed using UV-Vis spectrophotometry. This method is more biologically relevant and specific than other in vitro methods, as most inflammatory reactions involve protein denaturation and structural modification (8).

Materials and Methods

Materials

The materials used in this study included: *awar-awar* leaves obtained from the Central Metro area, Metro City, Lampung, 96% ethanol (JK Care®), Carbopol 940 (KJA®), Triethanolamine (TEA, KJA®), propylene glycol (KJA®), methylparaben (KJA®), aquadest, Hydrochloric Acid (HCl), Wagner's reagent, Dragendorff's reagent, Mayer's reagent, Liebermann-Burchard reagent, chloroform, Ferric Chloride ($FeCl_3$), Magnesium (Mg) powder, Amyl Alcohol ($C_5H_{12}OH$), glacial acetic acid, diclofenac sodium gel (Product X), Bovine Serum Albumin (BSA, HIMEDIA®), Tris Base (HIMEDIA®), and Sodium Chloride (NaCl).

Methods

Plant Identification

Plant determination was carried out at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), University of Lampung.

Preparation of Simplicia

Awar-awar leaves first underwent a wet sorting process to separate them from other parts, such as stems or twigs. After that, the sorted leaves were then washed under running water to remove dirt, and cut into small pieces to facilitate the drying process. Samples were oven-dried at 40°C for 24 hours (9).

Extraction Process

Maceration was performed using the dried *awar-awar* leaves and 96% ethanol as a polar solvent, in a sample-to-solvent ratio of 1:10. The mixture was stirred occasionally during the first 6 hours, then left undisturbed for the remaining 18 hours. The process was repeated (remaceration) using the same solvent, but at half the original volume. The resulting liquid extract was evaporated with a rotary evaporator (IKA RV 8 V-C) to remove the solvent and obtain a thick extract. The extract yield was calculated using the following formula (10):

$$\text{Yield (\%)} = \frac{\text{Weight of Extract Obtained}}{\text{Weight of Simplicia Used}} \times 100\% \quad \text{.....(1)}$$

Phytochemical Screening

Phytochemical screening is employed to identify the chemical compounds present in extracts through various tests. The alkaloid test is conducted by adding Mayer's, Wagner's, and Dragendorff's reagents to the extract solution. A yellow, brown, or orange precipitate indicates a positive result. The flavonoid test involves a reaction with Mg powder, amyl alcohol, and concentrated HCl. A red, yellow, or orange colouration indicates the presence of flavonoids. The polyphenol test uses a 1% solution of $FeCl_3$. A dark blue or blackish-green colour indicates a positive result. The saponin test is considered positive when stable foam forms after the solution is shaken with 2N HCl. The terpenoid and steroid tests employ Liebermann-Burchard reagent. A reddish-brown ring indicates triterpenoids, while a blue or green ring indicates steroids (11).

Gel Formulation

The gel formulations of *awar-awar* leaves extract are shown in **Table 1**.

Table 1. Gel Formulation of *Awar-awar* Leaves Extract

Materials	Concentration (% w/v)				Function
	F0	F1	F2	F3	
Awar-awar Leaves Extract	-	1.5	3	4.5	Active Ingredient
Carbopol 940	1	1	1	1	Gelling Agent
TEA	0.5	0.5	0.5	0.5	Alkalising Agent
Propylene Glycol	15	15	15	15	Humectant
Methylparaben	0.04	0.04	0.04	0.04	Preservative
Aquadest (Distilled Water)	83.46	81.96	80.46	78.96	Solvent

The manufacturing procedure began by dispersing Carbopol 940 in 50 mL of hot aquadest and stirring it for 10 minutes (Mixture I). Methylparaben was dissolved in propylene glycol until dissolved (Mixture II), then slowly added to Mixture I until it thickened. Next, the *awar-awar* leaves extract, and TEA were added, followed by the addition of the remaining aquadest and stirred until homogeneous. The gel was stored in a closed container and subjected to physicochemical evaluations and stability testing.

The Cycling Test

The cycling test was conducted over six storage cycles. Each cycle consisted of storage at 4±2°C for 24 hours, followed by transfer to an oven at 40±2°C for 24 hours. Evaluation of the preparation was carried out at cycle 0 and cycle 6, including organoleptic tests, determination of pH, viscosity, spreadability, and adhesiveness. The organoleptic test was conducted by visually observing the appearance, colour, and odour. Homogeneity was evaluated using two glass slides to ensure the absence of coarse particles or inhomogeneity. The pH was measured using a pH meter, with recommended values ranging from pH 4 to 8 (11). Viscosity was measured using a Brookfield viscometer (Viscometer IKA Germany ROTAVISC Hi-Vi I Complete), within the range of 2,000-50,000 mPa·s (12). Spreadability was assessed by measuring the diameter of the spread of 0.5 g of gel between glass plates, with a required range of 5-7 cm (13). Adhesiveness was evaluated by applying the gel between weighted glass slides, with an ideal adhesion time exceeding 1 second (12).

Preparation of Tris Buffer Saline (TBS) Solution

A total of 605 mg of Tris Base and 4.35 g of NaCl were dissolved in distilled water to a total volume of 400 mL. The pH was adjusted to 6.2-6.5 using glacial acetic acid. The solution was then brought to a final volume of 500 mL in a volumetric flask (15).

Preparation of 0.2% BSA Solution

100 mg of Bovine Serum Albumin (BSA) was accurately weighed and dissolved in TBS solution to a final volume of 50 mL in a volumetric flask (15).

Anti-Inflammatory Activity Testing

Each sample of *awar-awar* leaves extract gel (F1, F2, F3) and 1% diclofenac sodium gel was weighed and then dissolved in aquadest. Serial dilutions were prepared at concentrations of 200, 400, and 800 µg/mL. 0.5 mL of each concentration of the samples was mixed with a 0.2% BSA solution to a volume of 5 mL. Aquadest was used as the negative control. Each solution was incubated for 15 minutes at 37°C, followed by heating at 70°C for 5 minutes. After cooling, the solution was shaken vigorously to prevent agglomeration and to facilitate reading using a spectrophotometer. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 660 nm, and the percentage of inhibition was calculated. Samples were considered to exhibit anti-inflammatory activity when the rate of inhibition exceeded 20% (8). The inhibition percentage was calculated using the following formula (16):

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance of Negative Control - Absorbance of Test Solution}}{\text{Absorbance of Negative Control Solution}}\right) \text{x } 100\% \quad \text{..... (2)}$$

Data Analysis

Data were analysed using IBM SPSS version 26 software. Initial statistical testing involved the *Shapiro-Wilk* normality test and *Levene's* test for homogeneity, which were used to verify normal distribution and equal variance. A p-value greater than 0.05 indicated that the data met the assumptions of normality and homogeneity. If the data were normally distributed, a comparative test was conducted using *one-way ANOVA* (parametric). If the data were not normally distributed, the *Kruskal-Wallis* test (non-parametric) was applied. The results were considered statistically significant if the p-value was less than 0.05, and not significant if it was greater than 0.05. A P*aired Sample T-test* (parametric) was used to compare two groups or treatments and to assess their effect. In this study, the *Paired Sample T-test* was applied to compare cycle 0 and cycle 6 in the cycling test. A significant difference was reported if the p-value was less than 0.05. If the data were not normally

distributed, the *Wilcoxon* test was applied. The results were statistically significant if the p-value was less than 0.05. *Multiple linear regression* analysis was also used to determine the effect of independent variables on the dependent variable. This analysis was applied in the anti-inflammatory activity test. A meaningful relationship was indicated by a p-value less than 0.05 (17,18).

RESULTS

The use of colour reagents or qualitative testing in phytochemical screening facilitates the identification of secondary metabolite compounds in plants (19). The results of the phytochemical screening for *awar-awar* leaves extract are presented in **Table 2**.

Table 2. Phytochemical Screening Results of *Awar-awar* Leaves Extract

Test	Reagent	Result
Alkaloids	Wagner's reagent	Brown precipitate (+)
	Mayer's reagent	White precipitate (+)
Flavonoids	Mg, HCl, $C_5H_{12}OH$	Yellow solution (+)
Polyphenols	$FeCl_3$	Purple-black solution (+)
Saponins	Aquadest and shaken	No foam (-)
Triterpenoids/Steroids	Liebermann-Burchard	Blue solution (+)

(+) Indicates the presence of the compound; (–) Indicates the absence of the compound

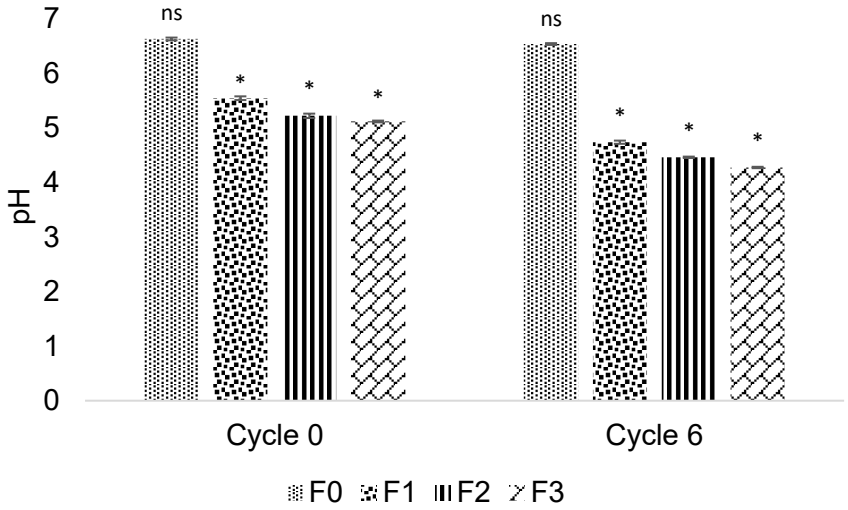

Figure 1. pH values of gel formulations (F0-F3) containing *awar-awar* leaves extract at cycle 0 and cycle 6 of the cycling test. * indicates a significant difference ($p<0.05$) between cycle 0 and cycle 6 within the same formulation; ns = not significant ($p>0.05$)

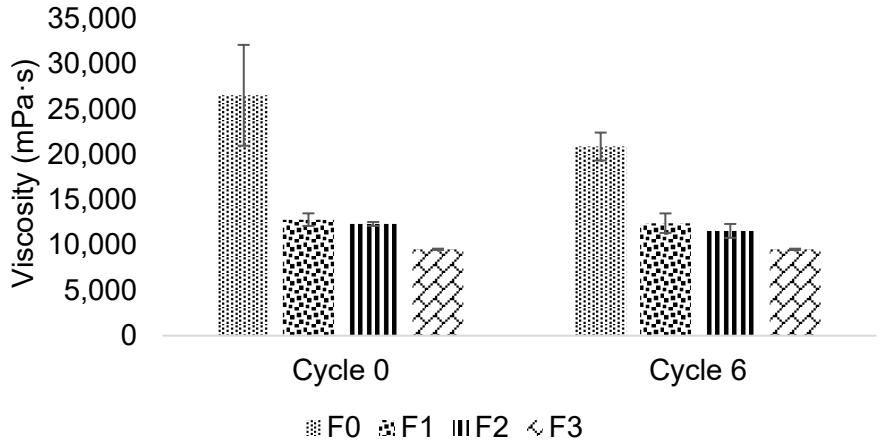

Figure 2. Viscosity values of gel formulations (F0-F3) containing *awar-awar* leaves extract at cycle 0 and cycle 6 of the cycling test. No statistically significant difference was observed between formulations in each cycle ($p>0.05$)

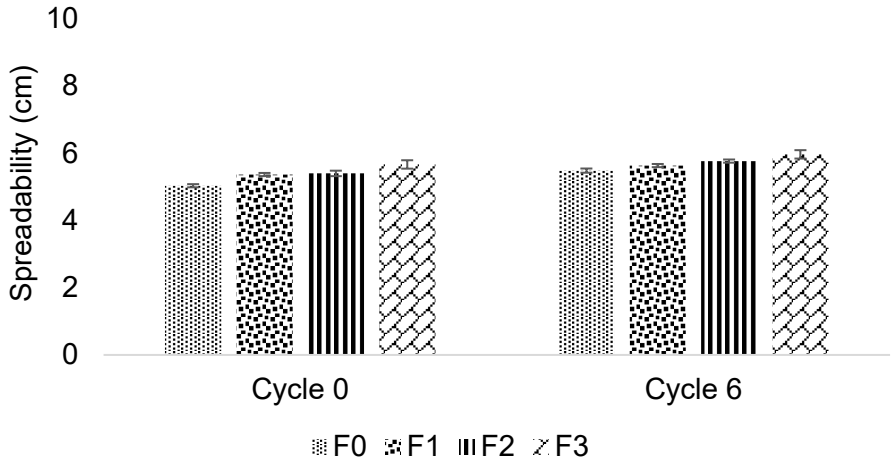

Figure 3. Spreadability values of gel formulations (F0-F3) containing *awar-awar* leaves extract at cycle 0 and cycle 6 of the cycling test. No statistically significant difference was observed between formulations in each cycle ($p>0.05$)

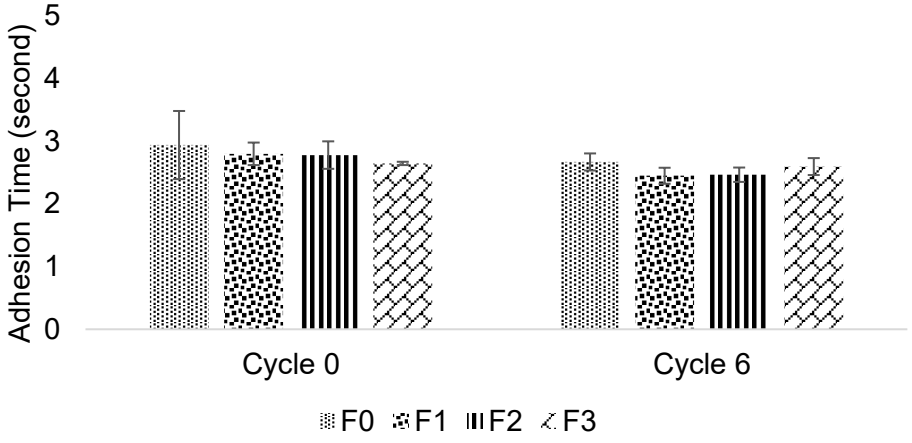

Figure 4. Adhesion time of gel formulations (F0-F3) containing *awar-awar* leaves extract at cycle 0 and cycle 6 of the cycling test. No statistically significant difference was observed between formulations in each cycle ($p>0.05$)

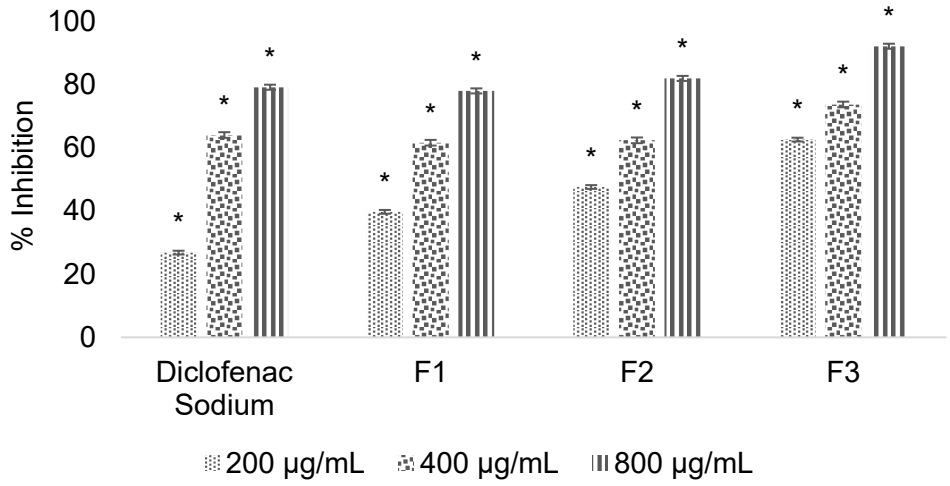

Figure 5. Percentage of inhibition of protein denaturation by *awar-awar* leaves extract gel formulations (F1-F3) at concentrations of 200, 400, and 800 µg/mL, compared to diclofenac sodium as a positive control. * indicates a significant difference ($p<0.05$) either between formulations and the control or among concentrations within the same formulation

DISCUSSION

Based on the results in **Table 2**, the ethanol extract of *awar-awar* leaves contains secondary metabolites, namely alkaloids, flavonoids, polyphenols, and steroids. Previous research has reported that *awar-awar* leaves contain alkaloids, flavonoids, saponins, polyphenols, triterpenoids, and steroids (20). However, in this study, saponin compounds were undetected. This difference may be attributed to variations in method, temperature, type of solvent, and extraction time (21). Polar solvents such as ethanol are more efficient at extracting polar compounds (19). Meanwhile, a longer extraction time increases the extract yield due to the prolonged contact between the material and the solvent. Elevated extraction temperatures may damage thermolabile compounds (8). The phytochemical content is also influenced by environmental factors at the growing site, such as temperature, light intensity, humidity, pH, and soil quality, all of which can affect the composition of plant secondary metabolites (22,23). The absence of saponins in this extract might affect its anti-inflammatory activity. However, anti-inflammatory effects do not solely rely on the presence of saponins. They are also influenced by other secondary metabolites still detected in the extract, such as flavonoids and polyphenols, which are known to possess anti-inflammatory properties. These compounds inhibit pro-inflammatory cytokines and enzymes such as Cyclooxygenase-2 (COX-2), thereby reducing inflammation (24).

In the organoleptic evaluation of the preparation, no visible changes were observed in colour, odour, shape, or texture. The results showed that F0 appeared clear, odourless, and had a gel-like texture, while F1, F2, and F3 retained a green colour, exhibited a distinctive odour, and also possessed a gel texture. The resulting green colour is due to the inherent green hue of the *awar-awar* leaves extract, which intensifies with increasing extract concentration. This finding is consistent with previous research, which reported that a higher concentration of extract affects the intensity of the formulation's colour (25). The homogeneity test showed that formulations F0, F1, and F3 produced homogeneous formulations, whereas F2 was not homogeneous. This was due to the presence of extract clots formed during the gel preparation process, which affected the homogeneity. This may also have been caused by insufficient stirring speed during formulation (26).

According to **Figure 1**, the pH values of all formulations decreased in pH in the sixth cycle for each formulation. The results remained within the acceptable pH range for the skin, which is 4-8. A *Paired Sample T-test* was conducted; the p-value obtained for F0 was >0.05, indicating that there was no significant difference in the pH of the preparations. However, the p-values obtained for F1, F2, and F3 were <0.05, indicating a significant difference in the pH of those preparations. These pH changes may occur due to the influence of temperature, humidity, and storage duration (26). The higher the extract concentration, the lower the pH value, due to the degradation of complex phenolic compounds

into simpler ones (27). This is consistent with previous research, which stated that increasing the concentration of *awar-awar* leaves extract reduces the pH of the formulation and can lead to a decrease in the gelling capability of Carbopol 940 (28).

Based on **Figure 2**, a decrease in viscosity was observed in all formulations (F0-F3) by the sixth cycle. The viscosity values remained within the acceptable range, which is 2,000-50,000 mPa·s, according to SNI 16-4399-1996 (13). A *Paired Sample T-test* revealed p-value greater than 0.05, indicating that there was no significant difference in the viscosity of formulations F0, F1, F2, and F3 between cycle 0 and cycle 6.

Based on **Figure 3**, the spreadability values for all formulations were within the acceptable range for gel preparations (5-7 cm) (14). There was an increase in spreadability in the sixth cycle for each formulation. This increase in spreadability is inversely proportional to the decrease in viscosity. This finding is consistent with previous research, which stated that the lower the viscosity, the higher the spreadability, and vice versa (6). A *Wilcoxon* test was carried out; the p-value obtained was >0.05, indicating that there was no significant difference in the spreadability of formulations F0, F1, F2, and F3 between cycle 0 and cycle 6.

In the adhesion test based on **Figure 4**, the values indicate the adhesion of each formulation under the requirements of a good gel, which is >1 second (14). There was a decrease in adhesion in the sixth cycle for each formulation. The viscosity of the formulations also influences this. In the sixth cycle, the viscosity of all formulations decreased, leading to a reduction in adhesion, as the adhesion test is directly proportional to viscosity (29). This is consistent with previous research, which stated that the adhesion of a formulation is directly proportional to viscosity (12). A *Paired Sample T-test* was carried out; the p-value obtained was >0.05, indicating that there was no significant difference in the adhesion of formulations F0, F1, F2, and F3 between cycle 0 and cycle 6. It can therefore be concluded that each formulation exhibited good stability, as no changes were observed due to changes in temperature and storage duration.

Based on the results of the *Kruskal-Wallis* test, a p-value of 0.000 ($p<0.05$) was obtained, indicating a significant difference between the anti-inflammatory activity of the comparator (diclofenac sodium), F1, F2, and F3. Further analysis using multiple linear regression revealed that both the formula and concentration had a statistically significant relationship with the absorbance value ($p<0.05$). The R value of 0.922 indicated a robust correlation between the two independent variables and absorbance. The regression analysis showed that the regression coefficient of the formula concerning absorbance was -0.039 ($p=0.007$), suggesting that increasing the formulation used led to a decrease in absorbance. Similarly, the regression coefficient of test concentration on absorbance was -0.098 ($p=0.000$), indicating that higher concentrations resulted in lower absorbance values. These results suggest that both formulation and concentration significantly affect absorbance, thereby influencing anti-inflammatory activity. Furthermore, F1, F2, and F3 at all tested concentrations demonstrated anti-inflammatory activity, as the percentage of inhibition exceeded 20% (16).

Protein denaturation is a condition in which proteins lose their tertiary and secondary structures due to exposure to external agents, such as strong acids, strong bases, organic salts, organic solvents, or heat (30). Denatured proteins are often recognised as foreign by the immune system. This recognition triggers the release of inflammatory mediators, such as cytokines and prostaglandins, which play a crucial role in the inflammatory process. Consequently, the greater the extent of protein denaturation, the higher the likelihood of inflammation due to immune activation (31). This process of protein denaturation may be inhibited by the presence of secondary metabolites found in *awar-awar* leaves extract, which possess anti-inflammatory potential. The interaction between molecules in BSA and bioactive compounds in the extract can inhibit protein denaturation (32). These secondary metabolites inhibit heat-induced protein denaturation, thereby reducing the amount of denatured protein and resulting in decreased turbidity levels. The lower the turbidity, the greater the anti-inflammatory activity observed (16). The results demonstrated that increasing the concentration of *awar-awar* leaves extract led to a reduction in turbidity levels.

Flavonoids have anti-inflammatory activity and have been considered a new class of natural anti-inflammatory agents (24,33). Flavonoids can inhibit inflammation by blocking the pro-inflammatory enzyme COX, which facilitates the release of prostaglandins from arachidonic acid as inflammatory mediators (16,34). Flavonoids can scavenge free radicals and Reactive Oxygen Species (ROS) that often cause damage to proteins, thereby reducing oxidative stress and protecting proteins

from denaturation. Heat exposure can increase the production of ROS, such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, which contribute to oxidative stress. Oxidative stress can induce inflammation by stimulating the release of inflammatory mediators (35). Although this study did not measure flavonoid levels, previous research reported that *awar-awar* leaves extract contains approximately 6.39% w/w flavonoids (4). Flavonoids may also prevent protein denaturation by interacting directly with amino acids in the BSA protein.

This compound can bind to aromatic tyrosine residues, aliphatic threonine, and lysine, which play a role in maintaining the stability of protein structures and help prevent changes in protein conformation (36). The most effective dose of ethanol extract of *awar-awar* leaves in reducing the volume and diameter of oedema was 200 mg/kg body weight in male rats induced with carrageenan (37). This anti-inflammatory effect is believed to result from the presence of flavonoid compounds in the ethanol extract of *awar-awar* leaves, which act as anti-inflammatory agents by inhibiting neutrophil degranulation, COX and lipoxygenase enzyme activity, histamine release, and inflammatory cell accumulation (36).

CONCLUSION

The evaluation of the physicochemical properties of formulations F0, F1, and F3, including organoleptic characteristics, homogeneity, pH, viscosity, spreadability, and adhesion demonstrated results consistent with the characteristics and requirements of a good gel as stated in SNI 16-4399-1996. However, formulation F2 showed poor homogeneity, which was attributed to inadequate stirring speed and time, as well as the clumpy texture of the extract. During the cycling test, the pH of F1, F2, and F3 was found to be unstable during storage, although still within acceptable limits. The gel formulations containing *awar-awar* leaves extract (F1, F2, and F3) exhibited anti-inflammatory activity, with inhibition percentages exceeding 20%. The highest inhibition was observed in F3, reaching 92.20%, which contained 4.5% extract concentration. These findings indicate that higher extract concentrations result in greater anti-inflammatory activity.

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