ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

Jurnal Kefarmasian Indonesia
Available online at https://jkefarind.com/index.php/jki
Original Research Article
JKI

Formulation and Antioxidant Activity of Syrup Preparation Containing Saga Leaf Extract (*Abrus precatorius* L.)

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ARTICLE INFO

Article history:
Received 16 October 2024
Revised 20 January 2025
Accepted 14 February 2025
Published online 28 February 2025

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DOI: https://doi.org/10.22435/jki.v15i1.6695

Citation: Dewi BS, Utami SM, Sayyidah, Alamsyah SS, Rahmadani NT. Formulation and Antioxidant Activity of Syrup Preparation Containing Saga Leaf Extract (*Abrus precatorius* L.). Jurnal Kefarmasian Indonesia. 2025;15(1):70-79.

ABSTRACT

Saga leaf (*Abrus precatorius* L.) have tannins, saponins, alkaloids, and flavonoids that have functions as antioxidants. The plant belongs to the Fabaceae family and is widespread in the tropics and subtropics. Syrup is one form of pharmaceutical preparation used in providing drugs in liquid form so that it is easily drunk by patients, especially children. The purpose of this study was to obtain the best syrup preparation formulation physically and its antioxidant activity. Saga leaf are extracted in ethanol and then syrup preparations by adding saga leaf extract with concentration variations of 0.5% (FI), 1% (FII), and 2% (FIII). Evaluation of syrup preparations includes organoleptic, pH, homogeneity, and antioxidant activity test using DPPH (1,1-diphenyl-2-picrylhydrazyl) measured by UV-Vis spectrophotometer at wavelength 516 nm. The results showed that Formula III had the best results, namely clear dark brown organoleptic, slightly viscous shape, distinctive smell and slightly bitter taste, pH 6.15, homogeneous, and the preparation was stable in storage for 12 days and showed the best IC_{50} value of 116.94 g/mL compared to FI (132.65 g/mL) and FII (118.03 g/mL). The antioxidant activity of saga leaf extract is categorized as moderate. In conclusion, saga leaf can be formulated as a syrup preparation with an extract concentration of 2% in formula III showing effectiveness as the best IC_{50} value of 116.94 g/mL and the best formulation.

***Keywords*:** Antioxidant; *Abrus precatorius*; DPPH; Saga leaf; Syrup

INTRODUCTION

Damage to cells and tissues of the human body caused by a chain reaction of free radicals has become a major concern in pharmaceutical and health sciences. Free radicals are molecules that have one or more unpaired electrons, as a result they tend to be reactive and can damage various cell components, including proteins, lipids, and nucleic acids. The accumulation of free radical damage has been linked to various degenerative diseases, such as cancer, diabetes, and cardiovascular disease.[1,2]

Antioxidants are nutrients that can help provide protection to cells from free radical damage. Antioxidants are substances that can neutralize free radicals and stop their chain reaction. Antioxidants have gained widespread interest as free radical protectors. Well-known antioxidants include vitamins C and E and phenolic compounds. Compounds called antioxidants have the capacity to stop the chain reaction caused by free radicals in the body. This aims to provide protection to body cells from potential damage that may be caused by these free radical reactions.[3,4] Furthermore, antioxidants can also play a role by giving electrons to free radicals, making them stable and no longer reactive. Antioxidants can be

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ISSN: 2354-8770 (Electronic)

found in both natural and synthetic forms. Some examples of natural antioxidants that are safe for human consumption include vitamin C, vitamin E, and flavonoids. On the other hand, synthetic antioxidants such as BHT (Butyl Hydroxy Toluene) and BHA (Butyl Hydroxy Anisol) are no longer used because they have the potential to cause carcinogenesis.[5,6]

The ethanolic extract of *A. precatorius* contains a total phenolic content of 419.04 ± 4.76 mg GAE/g ± SD and a flavonoid content of 153.33 ± 6.66 mg GAE/g ± SD. Phenolic compounds are among the most abundant secondary metabolites found in plants. These compounds exhibit strong antioxidant properties and the ability to combat free radicals, offering numerous potential health benefits. As natural antioxidants, phenolic compounds assist in neutralizing free radicals in the body, a function that has been supported by various epidemiological studies. The presence of multiple hydroxyl groups and their specific arrangement within phenolic molecules enhances their capacity to scavenge free radicals. [7,8]. Formulations of saga leaf extract that have been carried out are toothpaste as an antibacterial in the mouth[9], hydrogel film patch preparations as antibacterial[10], ointments to treat wounds[11]. So, the formulation of extracts in the form of syrup preparations as antioxidants has not yet been done. The choice of syrup preparation is based on effectiveness and convenience of consumption. The sweet flavour and liquid form are easily accepted by a wide range of ages, increasing compliance. In addition, syrup facilitates dose adjustment and accelerates the absorption of active substances. With these advantages, saga leaf extract syrup is expected to be an effective, practical, and widely accepted preparation.[12]

One simple yet fairly accurate method for measuring antioxidant capacity is the DPPH (*1,1-diphenyl-2-picrylhydrazyl*) test. DPPH is a free radical compound that can cause oxidative damage to molecules in the body. The DPPH testing process involves interactions between the test compound and DPPH free radicals. When antioxidant compounds react with DPPH radicals, they donate unpaired electrons to neutralize the radicals, converting them into stable, unreactive molecules. The discoloration from purple to yellow, or the absence of color change indicates that an antioxidant reaction has occurred.[13]

In this regard, research has been conducted to develop a syrup preparation formulation containing saga leaves extract (*Abrus precatorius* L.) with the aim of evaluating the antioxidant activity of the preparation. Saga leaves have been known as one of the plants in which there are bioactive compounds, which can act as a potential source of antioxidant compounds.[14] This formulation was evaluated in terms of physical properties, including organoleptic assessment, pH measurement, and homogeneity evaluation, and then tested using the DPPH method at a wavelength of 516 nm. This research is expected to produce the best formulation with the highest antioxidant activity in saga leaves extract syrup preparations. The results of this research should contribute to the development of skin care products that are more effective and natural in protecting the skin from oxidative damage.

Thus, this study aims to explore the potential of saga leaves extract in syrup preparation formulations as an alternative to increase the body's antioxidant intake through daily consumption. In this context, this research can make an important contribution in the development of pharmaceutical products that can help maintain health and prevent diseases due to oxidative stress caused by free radicals. In addition, it can also support the development of natural products that are environmentally friendly and have the potential to be used in antioxidant therapy.

METHODS

Source of plant material

The plant material used in this study was Saga leaves sourced from South Tangerang, Banten. The identification and authentication of the leaves were conducted by Dr. Ratih Damayanti, Director of Scientific Collection Management at the National Research and Innovation Agency, Central Jakarta, under the ID B-8776/II.6.2/1R.01.02/6/2023.

Equipment and materials

The material used were Saga leaf extract (National Research and Innovation Agency),

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ISSN: 2354-8770 (Electronic)

sucrose (Merck), propylene glycol (Merck), methylparaben (Merck), melon essence, aquades, Vitamin C (Sigma), DPPH (Sigma).

The equipment used includes a mortar and stamper, analytical balance (CHQ-DJ series) DJ303A, hot plate, water bath (Memmert), pH meter (Morinome EZ-9908), pycnometer (Iwaki), and UV-Vis Spectrophotometer (Spectroquant Pharo 300).

Extraction of saga leaf

The extraction of *Abrus precatorius* leaves was performed using the maceration method. A total of 1000 grams of saga leaf powder was soaked in 5 liters of 70% ethanol for 72 hours, with occasional stirring, maintaining a ratio of 1:5. After 72 hours, the mixture was filtered using filter paper to separate the extract. The filtrate was then collected and evaporated using a rotary evaporator at 50°C until a concentrated extract was obtained and weighed.[14]

Phytochemical screening of saga leaf extract

Phytochemical screening was carried out to identify flavonoids, saponins, tannins, and steroids. Flavonoid detection was performed by mixing 1 mL of the extract solution with 2 mL of NaOH. Identification of saponins is done by mixing the extract with 5 mL hot water and shaken and then allowed to stand for 10 minutes. Identification of tannins is done by reacting 1 mL of extract solution with 2 mL of FeCl3 5%. Steroid identification is done by dissolving 2 mg of extract in ethyl acetate. The ethyl acetate layer was dripped on a drip plate and allowed to dry. After that, anhydrous acetic acid and concentrated sulfuric acid were dripped on the drip plate.[15]

Standardization of non-specific parameters

The standardization of non-specific parameters was conducted by measuring water content, loss on drying, total ash content, acid-soluble ash, and acid-insoluble ash.[15]

Formulation of saga leaf extract syrup

Based on the formulation of syrup preparation containing saga leaf extract (Table 1) this syrup is created by heating method. Preparation of tools and materials is carried out first, then calibrate on a 60 mL bottle. Next, aquadest is put into a beaker glass and heated using a hot plate to dissolve sucrose, extract, and propylenglycol. Once dissolved, the mixture is transferred into a mortar and then propyl paraben, methyl paraben, and ascorbic acid are added to it. This mixture is then crushed until it reaches good homogeneity. Next, the mixture is put into bottles until it reaches the specified calibration mark. Finally, add 1 drop of melon essence to the mixture and beat thoroughly so that it becomes homogeneous.

Physical evaluation of saga leaf extract syrup

The physical evaluation of syrup preparation was tested for 12 days while it was kept at room temperature. The five test parameters used to determine stability were consists of organoleptic test, pH test, and homogeneity test[15].

Table 1. Saga Leaf Extract Syrup Formulation

Material	Formula %(b/v)			Usability
	FI	FII	FIII	
Saga Leaf Extract	0,5	1	2	Active substances
Sucrose	38	38	38	Sweetener
Propylene glycol	15	15	15	Solvent
Methylparaben	0.18	0.18	0.18	Preservative
Propylparaben	0.02	0.02	0.02	Preservative
Melon Essence	1	1	1	Fragrance & Flavoring
Aquades	100	100	100	Solvent

Note: FI = 0.5% Saga leaf extract, FII = 1% Saga leaf extract, FIII = 2% Saga leaf extract

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ISSN: 2354-8770 (Electronic)

Organoleptic test

Organoleptical evaluation was carried out by visual observation of syrup preparation which were obtained through shape, color, smell and taste.[16]

pH test

pH evaluation was carried out on syrup preparation using a pH meter. Before use, the pH meter was calibrated with a standard buffer solution. Put the test sample in a beaker glass, then the electrode is dipped in the sample, and observed the pH score in the screen. In the context of syrup, the recommended pH ranges from 4 to 7.[16,17]

Homogeneity test

Observation were perfomed by smearing 1 g of the syrup preparation on the object glass, after which covered with another glass object, and observing whether or not there were coarse grains visual and lumps in the syrup preparation.[4]

Specific gravity test

Density determination is done using a pycnometer that has been cleaned and dried well. At room temperature, an empty pycnometer (w1) is measured in weight, then filled with distilled water, the outside of the pycnometer is wiped dry, and after that the weight is measured again (w2). The distilled water liquid that is in the pycnometer is discarded, and the pycnometer is completely drained. Next, the dried pycnometer is filled with syrup preparations and measured in weight (w3). The density of the liquid is calculated using a predetermined formula.[18]

$$\rho = \frac{w3-w1}{w2-w1} \text{ x specific gravity of water (g/mL)}$$

Note
ρ = Specific weight of syrup
w1 = Weight of empty pycnometer
w2 = Pycnometer weight + distilled water
w3 = Empty pycnometer weight + syrup

Evaluation of the antioxidant activity of saga leaf extract syrup

The evaluation of the antioxidant activity of Saga leaf extract syrup preparation was conducted by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The concentration of the sample was 100µg/mL each formulation for initial screening at 5, 10, 25, 50 and 100µg/mL. Vitamin C (0.5, 1, 2, 4 and 8µg/mL) was utilized as a standard for comparison, and 0.4 mM DPPH was used as a control. The test solution, control and vitamin C were all incubated at 37°C for 30 minutes. It was then pipetted into a UV-Vis Spectrophotometer cuvette. The control blank was made from ethanol and compared to vitamin C. The absorption was measured by UV-Vis Spectrophotometer with 515 nm wavelength. The IC_{50} value, which represents the sample's ability to inhibit 50% of the oxidation process, was used to estimate antioxidant activity. It was calculated by plotting a linear relationship between the concentration of the test solution (x-axis) and the percentage antioxidant activity (y-axis).[19]

$$Antioxidant\ activity\ (\%) = \frac{\text{Ab} - \text{As}}{\text{Ab}} \text{ x } 100\%$$

Ab is the absorbance of the blank (without sample) and As is the absorbance of the sample.

RESULTS AND DISCUSSION

The plant material used in this study was Saga leaves sourced from South Tangerang, Banten. The identification and authentication of the leaves were conducted by Dr. Ratih Damayanti, Director of Scientific Collection Management at the National Research and Innovation Agency, Central Jakarta, under the ID B-8776/II.6.2/1R.01.02/6/2023. The phytochemical screening identified the presence of secondary metabolites, including tannins, saponins, alkaloids, and flavonoids. (Table 2).

Non-specific parameter test findings included water content, Drying shinkage, Ash content, Acid soluble ash content, and Acid insoluble ash content. The water content value was 12,30%. This value is slightly higher than the standard which is ≤ 10%. High water content can be a medium for microbes to grow which will reduce the stability of the extract. The drying shrinkage value was 12.30%. This indicates the amount of water content and compounds lost during the drying process

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was 12.30%. A good requirement for drying shrinkage is less than 10%, as drying shrinkage also represents the evaporated water content. The ash content and acid soluble ash content obtained a value of 2.17%, while the acid insoluble ash content obtained was <0.02%. These results fulfil the WHO requirement of no more than 2%. This indicates that the extract contains little inorganic residue (ash) (Table 3).[14]

Table 2. Phytochemical screening of saga leaf extract

Parameter		Result	Description
Tannins		+	Forms a blackish-green colour
Saponins		+	Stable froth
Alkaloids	Dragendoff	+	Formed orange colour
	Mayer	-	Solution does not form white precipitate
	Wagner	+	Red colour solution formed
Flavonoids		+	Formed orange colour
Steroid/tripertenoid		-	No red-purple colour is formed

Table 3. Standardization of non-specific parameters

Testing	Test Result
Water content	12,30%
Drying shinkage	12,30%
Ash content	2,17%
Acid soluble ash content	2,17%
Acid insoluble ash content	0,02%

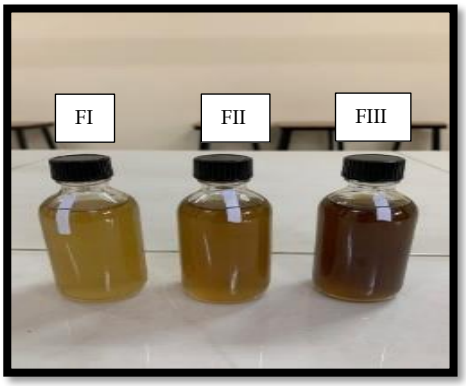

Figure 1. Syrup preparation containing saga leaf extract

Note:
FI : Saga leaf extract syrup formulation with a concentration of 0.5%
FII : Saga leaf extract syrup formulation with a concentration of 1%
FIII : Saga leaf extract syrup formulation with a concentration of 2%

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Table 4. Organoleptic test results of syrup preparation containing saga leaf extract

Time (Day)	Organoleptic	FI	FII	FIII
0	Shape	Liquid	Liquid	Liquid
	Color	Light Brown	Brown is a bit old	Dark brown
	Smell	Aromatic	Aromatic	Aromatic
	Taste	Sweet	A bit bitter	Bitter
3	Shape	Liquid	Liquid	Liquid
	Color	Light Brown	Brown is a bit old	Dark brown
	Smell	Aromatic	Aromatic	Aromatic
	Taste	Sweet	A bit bitter	Bitter
6	Shape	Liquid	Liquid	Liquid
	Color	Light Brown	Brown is a bit old	Dark brown
	Smell	Aromatic	Aromatic	Aromatic
	Taste	Sweet	A bit bitter	Bitter
9	Shape	Liquid	Liquid	Liquid
	Color	Light Brown	Brown is a bit old	Dark brown
	Smell	Aromatic	Aromatic	Aromatic
	Taste	Sweet	A bit bitter	Bitter
12	Shape	Liquid	Liquid	Liquid
	Color	Light Brown	Brown is a bit old	Dark brown
	Smell	Aromatic	Aromatic	Aromatic
	Taste	Sweet	A bit bitter	Bitter

The results of organoleptic test of saga leaf extract syrup preparations for 12 days show that the formula I (FI) has a distinctive aromatic smell of saga leaves a clear light brown color, and a sweet taste. Then the formula II (FII) also has a characteristic aromatic smell, a slightly translucent dark brown color, and a slightly bitter taste. As for formula III (FIII), it also has a characteristic aromatic smell, and translucent dark brown color (Table 2). This can be happen because FIII used higher concentraion of saga leaf extract compared to FI and FII so that the color becomes dark brown and bitter taste (Figure 1).[19]

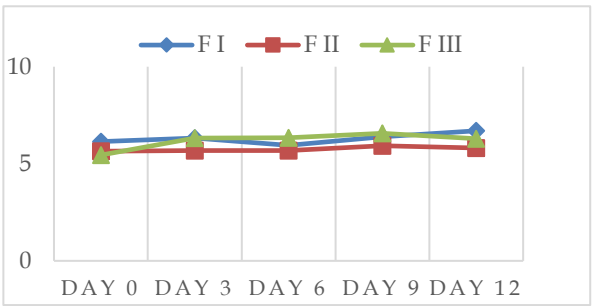

Figure 2. Curve of pH test results of syrup preparation containing saga leaf extract

Table 5. Homogeneity test results of syrup preparation containing saga leaf extract

Time (Day)	Homogeneity Test		
	FI	FII	FIII
0	Homogeneous	Homogeneous	Homogeneous
3	Homogeneous	Homogeneous	Homogeneous
6	Homogeneous	Homogeneous	Homogeneous
9	Homogeneous	Homogeneous	Homogeneous
12	Homogeneous	Homogeneous	Homogeneous

Homogeneity testing over the 12-day storage period of saga leaf extract syrup preparations, as listed in Table 5, showed that all formulations remained homogeneous with no insoluble particles in the preparation. This finding is by the requirements of syrup preparations that must remain homogeneous.[18]

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Table 6. pH test results of syrup preparation containing saga leaf extract

Time (Day)	pH		
	FI	FII	FIII
0	6.15	5.65	5.45
3	6.32	5.69	6.33
6	5.95	5.68	6.34
9	6.39	5.93	6.57
12	6.70	5.82	6.29
Mean	**6,90 ± 0.28**	**5,75 ± 0.12**	**6,19 ± 0.57**

The recommended pH for syrup preparations is 4-7.[18]

pH test results of syrup preparations containing saga leaf extract within 12 days of storage at room temperature with three variations in extract concentration is shown in Table 4 and Figure 2. This measurement aims to assess the stability of the preparation during the storage period. FI experienced a pH change from 6.15 on day 0 to 6.70 on day 12, with an average pH of 6.90. FII increased its pH from 5.65 on day 0 to 5.82 on day 12, with an average pH of 5.75. While FIII experienced a change in pH from 5.45 on day 0 to 6.29 on day 12, with an average pH of 6.19. This pH change may be caused by environmental factors such as temperature and less-than-optimal storage conditions. Although there are pH changes in all three formulations, the pH value in all formulations is still within a safe range, which is 4-7.[18]

The results of statistical analysis of the pH test of syrup preparations of formulas 1, 2, and 3 in the Shapiro-Wilk normality test showed that the data were normally distributed (p≥0.05). In the Homogeneity of Variances test, a significance value of 0.193 (p≥0.05) was obtained, indicating that the data had a homogeneous variance. In the parametric analysis of One Way ANOVA test, a significance value of 0.104 (p≥0.05) was obtained, indicating that there was no significant difference in the mean pH between the three formulas. The results of this statistical analysis indicate that the formula variation does not significantly affect the pH value, and the data is considered homogeneous.[25]

Table 7. Specific gravity test results of syrup preparation containing saga leaf extract

Specific gravity (g/mL)		
FI	**FII**	**FIII**
1.2775	1.2530	2.0972

The specific weight of a good syrup is 1.3 g/mL

Based on Table 5, the specific gravity test results of syrup preparations containing saga leaf extract obtained value of each different formula. The specific gravity value for FI is 1.2775, FII 1.2530, and FIII 2.0972. This finding aligns with Zakaria N et al. (2024), who reported that the specific gravity of SPKKJ falls between 1.021 and 1.028 g/mL, which meets the polyherbal syrup's specific gravity requirements of 1.02 to 1.19 g/mL.[20] Besides that, Herdaningsih S and Kartikasari D (2022) and Susanti SFE (2023) indicated that a desirable specific gravity for syrup preparations is greater than 1.2 g/mL.[21,22] Thus, it can be concluded that all three formulas satisfy the required specific gravity standards.

Figure 3 presents linear regression equations for each vitamin C type, with corresponding R2 values indicating strong correlations between the independent and dependent variables (R2 values range from 0.992 to 0.9997). The equations are as follows: Vitamin C I: y = 2.7083x + 13.294; Formulation I: y = 0.5056x – 17.07; Formulation II: y = 0.39x + 3.9679; Formulation III: y = 0.2577x + 19.864.

Table 8. Antioxidant activity analysis of syrup preparation

F	IC_{50} value (ppm)	Category
FI	132.65	Moderate
FII	118.03	Moderate
FIII	116.94	Moderate

Based on the data listed in Table 6, testing the antioxidant activity of syrup preparations has been carried out using the DPPH method (2,2 Diphenyl-1-Picryhydrazil) and measuring its absorbance by UV-VIS spectrophotometry at a wavelength of 514 nm. The method requires a small sample to perform antioxidant testing. The results show that FIII (2% saga leaf extract) has the lowest IC_{50} value of 116.94 ppm which

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ISSN: 2354-8770 (Electronic)

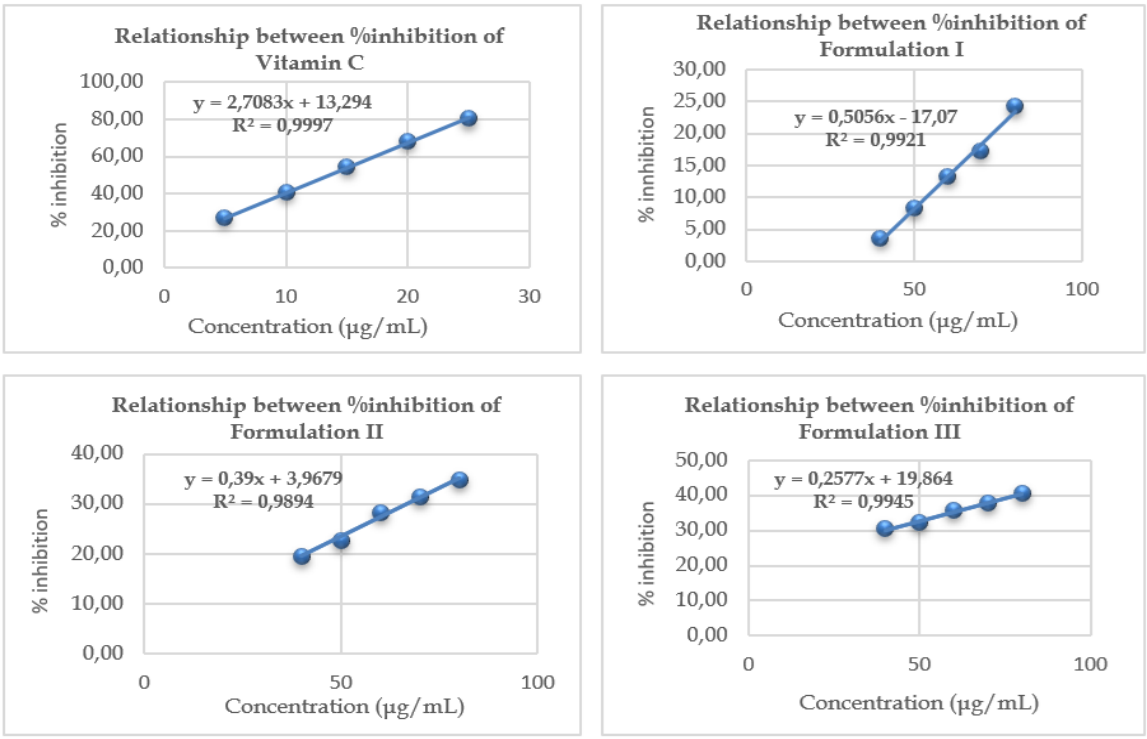

Figure 3. Relationship curve of antioxidant activity: vitamin C, formulation I, formulation III, and formulation III

means it has the highest antioxidant activity compared to FI (132.65 ppm) and FII (118.03 ppm) which are classified into the category of moderate antioxidant activity. This shows that the amount of saga leaf extract in each formula affects the value of antioxidant activity. The IC_{50} value is called very strong if it produces a value of less than 50 ppm, as in ascorbic acid (standard) in this study of 12 ppm.

Analysis of the aqueous leaf extract with GCMS has revealed the presence of numerous bioactive compounds with antioxidant properties. These include essential oils, flavonoids, and tannins, further confirming the plant's efficacy in neutralizing oxidative species.[23] The leaves and seeds of *Abrus precatorius L.* have been extensively studied for their rich phytochemical content, including flavonoids and polyphenols, which contribute to its potent antioxidant properties. The methanolic extracts of the leaves have been shown to exhibit strong free-radical scavenging activity, supporting its use in traditional medicine.[24]

Increasing the level of saga extract from 0.5% (FI), 1% (FII), to 2% (FIII) caused changes in organoleptic parameters, such as shape, colour, smell and taste. The formula with higher extract content (FIII) showed a darker colour, and a more bitter taste. The increase in saga extract content is directly proportional to the antioxidant activity. This is due to the higher flavonoid and phenolic content at higher extract concentrations, thus the ability to counteract free radicals increases.

CONCLUSION

The results showed that saga leaf can be formulated as a syrup preparation with an extract concentration of 2% in formula III showing effectiveness as the best IC_{50} value and the best formulation. Formula III had the best results, namely clear dark brown organoleptic, slightly viscous shape, distinctive smell and slightly bitter taste, pH 6.15, homogeneous, and the preparation was stable in storage for 12 days and showed the best IC_{50} value of 116.94 g/mL compared to FI (132.65 g/mL)

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

and FII (118.03 g/mL). The antioxidant activity of saga leaf extract is categorized as moderate.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

Acknowledgments

The authors are grateful to STIKes Widya Dharma Husada Tangerang for facilitating this research.

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