OPTIMIZATION OF CARBOPOL 940 AND GLYCEROL CONCENTRATION IN ANTIOXIDANT GEL OF *ALSTONIA SCHOLARIS* L. LEAF EXTRACT WITH SIMPLEX LATTICE DESIGN METHOD

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ABSTRACT

Alstonia scholaris L. contains several compounds, which has been proven to be potential as skin protection by formulating in gel preparation. The aims of this study was to obtained the concentration of carbopol 940 and glycerol to produce an optimal gel preparation of *Alstonia scholaris* L. leaf extract. Simplex lattice design was used as optimization method with two independent variables, carbopol 940 (0.5%-2.5%) as gelling agent and glycerol (14%-16%) as humectant. There were 5 formulas carried out to physical properties evaluation: organoleptic, homogeneity, spreadability, pH, adhesion time and viscosity. The result showed that the prediction of optimal formula from SLD method with 1.67% carbopol 940 and 14.83% of glycerol. The physical properties of optimal formula were 5.206±0,125 of pH value; 28,768.24±4,608.11 cP of the viscosity value; 5.96±0,087 cm of spreadability value; and 55.146±1.174 seconds of adhesion. There was no significant difference of SLD prediction responses with observation response.

Keywords: *Alstonia scholaris*, Antioxidant Gel, Simplex Lattice Design Method

INTRODUCTION

The *Alstonia scholaris* L. can be used medicinally to treat disease and have advantages over chemical drugs. Phytochemical studies have shown that the genus of Alstonia has monoterpenoid indole alkaloids (MIAs) as the most compounds isolated from this genus. The other components are flavonoids, phenolic acids, terpenes, volatile oils, etc (Zhao et al., 2023). *Alstonia scholaris* L. leaf extract had the highest content of total phenolic (49.66±1.52 mg GAE/g) followed by follicle (18.68±1.53 mg GAE/g) and latex extracts (17.0±2.0 mg GAE/g). total flavonoids and proanthocyanidins content of *Alstonia scholaris* L. leaf were present in abundance than follicles and latex part with value observed 97.33±1.52 mg QE/g (flavonoids) and 99.33±1.52 mg CE/g (proanthocyanidins). *Alstonia scholaris* L. was approved had good pharmacological effects, including high scavenging activites with inhibition percentage value 70-80% and IC_{50} value of methanolic leaf extract was 60.9 ppm (Ganjewala & Gupta, 2013; Shanmugapriya & Jayanthi, 2019).

Our present study, *Alstonia scholaris* L. methanolic extract from leaves part was dispersed in gel preparation for topical application to protect skin. Topical application of drug still be the best route for skin demage caused by free radicals, site specific drug delivery, and minimize side effects (Bharadwaj et al., 2019). The physical properties and stability of gel preparation influenced by the selection and concentration of gelling agent and humectant (Indah et al., 2023). Carbopol are acrylic acid polymers crosslinked with divinyl glycol or polyalkenyl ethers. Carbopol are non-irritant and non-toxic excipient with not proven of hypersensitivity as topical preparation (Das et al., 2013).

Different types of Carbopol have different viscosity properties depending on their particle size, distributions of cross-links, molecular weight between cross links and free chain ends. Carbopol 940 has 1450 monomer unit or high molecular weight with its value 104,4 g/mole. Carbopol 940 was selected as gelling agent because its good consistency, not sticky and clear appearance (Indah et al., 2023). Glycerol as humectant was selected to preserve the stability of gel preparation by minimizing water evaporation. Aside, humectant can keep skin wet and prevent it from drying out (Vityazev et al., 2020).

In order to make the optimal physical properties of gel preparation, optimization of excipient concentration should be done using simplex lattice design method which fast, easy, and widely used to optimize the mixture of ingredient compared trial and error method. this method used to determine the relative proportions of excipient for produce the best formula of gel preparation with constant weight (Pratiwi et al., 2020).

RESEARCH METHODS

Materials

Alstonia scholaris L.'s methanolic extract, aquadest, carbopol 940, glycerol, methyl paraben, propyl paraben, sodium hydroxide.

Gel Formula

Gel preparation was made follow the formula below:

Table 1. Formula of *Alstonia scholaris* L.'s Methanolic Extract Gel Preparation

Ingredients	Function	Concentration (%)
Alstonia scholaris L.'s methanolic extract	Active ingredient	1
Carbopol 940	Gelling agent	0.5 – 2.5
Glycerol	Humectant	14 – 16
Methyl paraben	Antibacterial	0.18
Propyl paraben	Antifungi	0.02
Sodium hydroxide	Alkylating agent	0.7
Aquadest	Solvent	Ad 100

Gel Preparation

Gel was prepared by dissolved methyl paraben and propyl paraben with glycerin while it heated on a hotplate until homogeneous. *Alstonia scholaris* L.'s methanolic extract was dissolved with previous mixture using a homogenizer for about 20 minutes (mixture 1). Next, Carbopol was dissolve with aquadest by sprinkling it on top of solvent in mortar, stir, and left at room temperature for 24 hours to allow it expand (mixture 2). Mixture 1 and sodium hidroxide was added into mixture 2 then added aquadest until 100 mL, stir until homogeneous.

Physical properties

Organoleptic

The organoleptic test of gel preparation was carried out visually by observations the smell, color, and texture (Zaky et al., 2021).

Homogeneity
The homogeneity test of gel preparation was carried out visually by 1 gram was placed on a petri dish and then covered with the bottom of the other petri dish. A good preparation is characterized by the absence of coarse particles in the gel preparation (Fauziah et al., 2020).

Spreadability
Gel preparation (1 gram) placed on a glass scale, covered with other glass. Then, 50 g and 100 g load was placed on top for 1 minute. The diameter of the spread was measured by vernier caliper (Zaky et al., 2021).

Adhesion time
The test was carried out by placing 0.5 gram of gel preparation on an object glass, then placing another object glass on top of the gel and pressing it with a 1 kg of load for 5 minutes. The adhesion time was the time of object glass separated after load released (Zaky et al., 2021).

pH evaluation
a define amount of gel preparation was measured pH value using pH meter by bringing it in direct contact and allowing it for one minute or until the number of pH value on the screen stay still. All pH measurements were performed in triplicate (Fauziah et al., 2020).

Viscosity measurements
The viscosity value of gel was measured by Brookfield viscometer with appropriate spindle and rotation rate. The spindle has to covered by gel preparation for the measurement. All viscosity measurements were performed in triplicate (Fauziah et al., 2020).

Data analysis
The optimal formula was obtained based on viscosity, adhesion, spreadability, and pH response with a desirability value target close to 1. The optimal formula was carried out a verification test by comparing the predicted response from the experimental design software to the real results used T-Test. No significant difference of T-Test result indicate that the equation from simplex lattice design method is valid (Pratiwi et al., 2020).

RESULT AND DISCUSSION

This research used DoE (Design of Experimental) Software version 13 with simplex lattice design method considered 5 formulas (table 2) to determine composition of mixture with the best physic properties of gel preparation as optimum formula. The compositions, materials or variables used in optimization consist at least two types. This study was used two variables independent. They were carbopol as gelling agent and glycerol as humectant.

Table 2. Formula of *Alstonia scholaris* L.'s methanolic extract Gel Preparation

Ingredients	Formula (%)				
	1	2	3	4	5
Alstonia scholaris L.'s methanolic extract	1	1	1	1	1
Carbopol	1.5	2	1	0.5	2.5
Glycerol	15	14.5	15.5	16	14
Methyl Paraben	0.18	0.18	0.18	0.18	0.18
Propyl Paraben	0.02	0.02	0.02	0.02	0.02
Sodium Hydroxide	0.7	0.7	0.7	0.7	0.7
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

The result of physical properties of gel preparation (organoleptic and homogeneity) shown in table 3 and the viscosity, pH, adhesion time, and spreadability shown in table 4.

Table 3. Organoleptic and Homogeneity Properties of Gel Preparation

Formula	Consistency	Smells	Color	Homogeneity
1	slightly thick	distinctive smell of leave	Dark green	Homogeneous
2	thick	distinctive smell of leave	Dark green	Homogeneous
3	slightly thick	distinctive smell of leave	Dark green	Homogeneous
4	liquid	distinctive smell of leave	Dark green	Homogeneous
5	Very thick	distinctive smell of leave	Dark green	Homogeneous

From the table above, the homogeneity results shown that all formulas are homogeneous as indicated by there's no powder particles, no change in colour and smell in gel preparation. This illustrates that the extract and ingredients used are mixed well. According to the literature, a homogeneous preparation guarantees a uniform amount of active substance when taken (Putri & Anindhita, 2022).

Table 4. Physical Properties of gel preparation

Formula	pH ± SD	Viscosity ± SD (cP)	Spreadability ± SD (cm)	Adhesion time ± SD
1	5,45 ± 0,092	15133,50 ± 3509,357	5,77 ± 0,853	50,88 ± 3,589
2	5,11 ± 0,045	30620,85 ± 6255,604	5,37 ± 0,267	60,86 ± 4,431
3	5,53 ± 0,088	11264,42 ± 3361,216	6,23 ± 0,563	33,94 ± 2,036
4	5,84 ± 0,065	4927,71 ± 1921,534	6,74 ± 0,699	21,15 ± 2,002
5	4,93 ± 0,064	54178 ± 26520,036	5,59 ± 0,221	79,24 ± 7,662

equation	pH = -0,081697x + 0,366303y	Viscosity = 22836,37224x – 735,30176y	spreadability = -0,214545x + 0,417455y	Adhesion time = 29,00085x +0,380848y

X = Carbopol concentration (%)
Y = glycerol concentration (%)

pH value was carried out to see the acidity level of the gel preparation. The pH for topical preparations that are safe on the skin is in the range 4.5 - 6.5. If the pH value of gel preparation is low (acidic), it can irritate the skin, while its too high (alkaline), it can cause dry skin [12]. The table above shown that the pH changes were still in the skin pH range. The use of Carbopol can change the pH value of the preparation due to the chemical reaction of the carboxylate group with water to form H3O+ which is acidic (Yuliandari et al., 2021).

The different viscosity values in each formula were due to differences in the composition of gelling agent (Carbopol). It can be seen in tables that F1, F2, F3, and F5 has viscosity values in standard range for gel preparations (6000-50000 cP). Meanwhile, F4 viscosity value is smaller among all formulas, it was because of less carbopol concentration. Enhancing the quantity of gelling agent can strengthen the gel matrix, caused the viscosity value increase as the concentration of carbopol rises. Aside from that, another reason low viscosity of gel preparation was the polymer's influence on temperature variations. When a gel is stored at high temperatures, the polymer chains uncoil into a spherical shape (disentangle), caused the gel's viscosity decrease (diluting). Meanwhile, if a gel is stored at freezing temperatures, the polymer chains shorten and gel preparation entangle (Mursyid, 2017).

The spreadability test aims to determine the ability of gel preparation to spread evenly on the skin surface. Good spreadability is in range of 5-7 cm [15]. good spreadability can make gel preparation applied easily without excessive pressure and it can make wider surface area of the gel is applied to the skin. The spreadability test shown that five formulas were in accordance with the standard spreadability test range, the highest spreaddability value was formula 4 with 6.74 cm which had the least amount of carbopol with a greater amount of glycerin. The viscosity of gel preparation was inversely proportional to the resulting spreadability. The result shown that the enhancement of carbopol concentration with low concentration of glycerol caused a smaller value of spreadability (Hidayati et al., 2022).

The aim of conducted the adhesion time was carried out the time of gel preparation stick to the skin. The adhesion ability to the skin is expected to be able to provide a moisturizing effect on the skin. The good time criteria for sticking power is more than 10 seconds. The longest of adhesion time was on F5 with 79.24 seconds with the most carbopol. The fastest adhesion time was F4 with the least amount of carbopol.

From each response, the criteria of optimal formula were determined, such as maximize, minimize, in range, target or equal. From the target, DoE were predicted the composition of carbopol and glycerol; and predicted value of respons (pH, viscosity, spreadability, and adhesion time). The criteria and predicted value of respons shown in table 5. From the criteria, formula optimal was shown with percentage of Carbopol 1.67% and glycerol 14.83% with desirability value 0.441. The desirability value is a value that shows the DoE software's ability to predict optimum formula based on the specified response criteria. A desirability value which is close to 1 shows the ability of the software to produce products that meet the desired targets (Ramadhani et al., 2017).

Table 5. The criteria of optimum formula and prediction of respond value

Respons	Target	Range value	Prediction value	Experiment value
pH	maximize	4.5 – 6.5	5.296	5.206
Viscosity (cP)	Maximize	6000 – 50000	27242.104	28768.24
Spreadability (cm)	Maximize	5.37 – 6.74	5.832	5.96
Adhesion time (seconds)	maximize	19.2 – 79.24	54.091	55.146

The formula optimal of gel preparation was made with recommendation concentration of carbopol and glycerol from DoE before and evaluated the physical properties. The prediction value of physical properties (responds) from software were compared with the experiment respond value using one sample T-Test analysis to determine the significance differences (Suryani et al., 2017). The result shown with p value >0.05 means there were no significant difference between prediction respond and experimental respond and the equation from SLD method was valid.

CONCLUSION

The higher of the concentration of Carbopol can increase viscosity and adhesion time also decrease of pH and spreadability value of *Alstonia scholaris* L.'s metabolic extract gel preparation. From this research has shown that simplex lattice design using Design of Experimental software can have predicted the optimal formula validly.

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