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Original Article

Optimalisation of tablet formula from chlorophyll of *Sauropus androgynus* (L.) Merr. leaves using simplex lattice design (SLD)

[Optimización de la fórmula de comprimidos a partir de clorofila de hojas de *Sauropus androgynus* (L.) Merr. mediante diseño reticular simple (SLD)]

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Abstract

Context: *Sauropus androgynus* (L) Merr, known as a sweet leaf, contains chlorophyll, potentially an antioxidant. The selection of appropriate excipient composition affects the physical properties and therapy effectiveness of tablets from *S. androgynus* chlorophyll.

Aims: To determine the composition of the optimum excipient with variations in magnesium stearate and microcrystalline cellulose (MCC) concentrations and evaluate the physical properties of the tablet formula of chlorophyll *S. androgynus* leaves.

Methods: *S. androgynus* chlorophyll tablets were formulated using the direct compression method. The dose of *S. androgynus* chlorophyll was converted from the effective dose of antianaemia in rats of 0.016 mg/200 g BW to the human dose of 0.896 mg. A simplex lattice design (SLD) was adopted to optimize the eight formulations.

Results: SLD can be used to optimize the concentrations of magnesium stearate and MCC in the tablet formulation from *S. androgynus* chlorophyll. The 5% magnesium stearate formula and 20% MCC had the highest uniformity and disintegration time. The relative concentration of magnesium stearate and MCC influence the thickness, weight, hardness, friability, and disintegration time of the tablets produced.

Conclusions: The 5% magnesium stearate formula and 20% MCC had the shortest disintegration time and can be considered the optimum formula for tablets from *S. androgynus* chlorophyll. Further study on the beneficial and toxic effects of tablets from *S. androgynus* chlorophyll would be very beneficial in preventing iron deficiency anaemia.

Keywords: chlorophyll; magnesium stearate; microcrystalline cellulose; simplex lattice design; tablet.

Resumen

Contexto: *Sauropus androgynus* (L) Merr, conocida como hoja dulce, contiene clorofila, un antioxidante potencial. La selección de la composición apropiada del excipiente afecta las propiedades físicas y la efectividad terapéutica de los comprimidos de clorofila de *S. androgynus*.

Objetivos: Determinar la composición del excipiente óptimo con variaciones en las concentraciones de estearato de magnesio y celulosa microcristalina (MCC) y evaluar las propiedades físicas de la fórmula de comprimidos de clorofila de hojas de *S. androgynus*.

Métodos: Se formularon comprimidos de clorofila de *S. androgynus* utilizando el método de compresión directa. La dosis de clorofila de *S. androgynus* se convirtió de la dosis efectiva de antianemia en ratas de 0,016 mg/200 g de peso corporal a la dosis humana de 0,896 mg. Se adoptó un diseño de red simplex (SLD) para optimizar las ocho formulaciones.

Resultados: La SLD se puede utilizar para optimizar las concentraciones de estearato de magnesio y MCC en la formulación de comprimidos a partir de clorofila de *S. androgynus*. La fórmula de estearato de magnesio al 5% y MCC al 20% tuvo la uniformidad y el tiempo de desintegración más altos. La concentración relativa de estearato de magnesio y MCC influye en el espesor, el peso, la dureza, la friabilidad y el tiempo de desintegración de los comprimidos producidos.

Conclusiones: La fórmula de estearato de magnesio al 5% y MCC al 20% tuvo el tiempo de desintegración más corto y puede considerarse la fórmula óptima para comprimidos a partir de clorofila de *S. androgynus*. Un estudio adicional sobre los efectos beneficiosos y tóxicos de los comprimidos a partir de clorofila de *S. androgynus* sería muy beneficioso para prevenir la anemia por deficiencia de hierro.

Palabras Clave: celulosa microcristalina; clorofila; comprimido; diseño reticular simplex; estearato de magnesio.

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INTRODUCTION

Sauropus androgynus (L) Merr or *Breynia androgyna* (L.) Chakrab. & N.P.Balakr, known as sweet leaf, is widely used as a medicinal plant from the family *Phyllanthaceae*. In Indonesia, the plant known as *katuk* is widely used for increasing breastmilk production (Rosa et al., 2022). Dark green *S. androgynus* leaves contain many vitamins, nutrients, and active compounds, including carbohydrates, glycosides, saponins, proteins, tannins, flavonoids, steroids, alkaloids (Kwartiningsih et al., 2015), and chlorophyll (Suparmi et al., 2016a; 2016b). *S. androgynus* leaf chlorophyll showed antianaemia effects in sodium nitrite-induced mice (Suparmi et al., 2016a; 2016b). Previous research by Suparmi et al. (2016b) reported that *S. androgynus* chlorophyll at a dose of 0.016 mg/200 g BW, reduced the percentage of schistocytes, increased haemoglobin levels, and increased ferritin levels in sodium nitrite-induced rats. *S. androgynus* chlorophyll treatment has the potential as a new herbal supplement to cure diabetes mellitus and iron deficiency anaemia, although the safety dose should be considered to prevent toxicity (Suparmi et al., 2021). However, previous studies have used chlorophyll as a liquid, which may affect its stability. Tablet formulation can be the alternative to improve the stability of *S. androgynus* chlorophyll and be used as an antianaemia supplement.

Tablets are widely accepted standard dose forms, accounting for 50-60% of all dosage forms. Tablets remain the most preferred dosage amongst all the oral dosage forms due to their greatest ability to deliver a precise amount, lowest cost, compact design, ease of manufacture, and best-combined properties of chemical, mechanical, and microbiological stability (Sharma et al., 2015). Direct compression is the most widespread technique in tablet formulation to improve the consistency of chlorophyll as an active pharmaceutical ingredient (API). Appropriate excipient composition affects the rate of API release in the gastrointestinal tract to maximize the therapeutic outcomes of the produced tablet (Satish et al., 2012; Shanmugam, 2015).

Excipient compositions can be formulated using statistical designs, including simplex lattice design (SLD), which can assess excipients' influence, proportions, and processing. SLD is a viable tool for predicting the drug release patterns of extended-release formulations (Phadke et al., 2019). The designs can further enhance the formulation composition and processing circumstances for the intended target product profile (Kincl et al., 2005; Lestari et al., 2020). This research was conducted to optimize the concentration of magnesium stearate and microcrystalline cellulose (MCC) of avicel PH 101 as the excipients in the tablet formulation of *S. androgynus* chlorophyll using SLD.

MATERIAL AND METHODS

Chemicals

All chemicals were of analytical grade. Fresh leaves of *S. androgynus* were collected from the resident park in Perumahan Sembungharjo Permai, Genuk, Semarang, Central Java, Indonesia at the Global Positioning System (GPS) coordinates of -6.97, 110.48. The plant species (No. 107/UN27.1.4.5/LT/2023) was authenticated by a taxonomist at the Department of Biology, Universitas Negeri Semarang, Indonesia

Extraction of chlorophyll from *S. androgynus* leaves

A given amount (100 g) of fresh leaves was cleaned and blended with distilled water using an electric blender until the pigment was removed. The extract was filtered to remove insoluble material. The filtrate obtained was dried using a freeze-dryer. The weight difference in fresh and dry weights was determined by the water content of the chlorophyll extract.

Determination of chlorophyll A and B

The levels of chlorophyll A and B were quantified using a spectrophotometer (UV-Vis Agilent Thermo Scientific, Agilent Technology.ink, USAs). The optical density (OD) was set at wavelengths of 645 and 663 nm, respectively (Dharmadewi, 2020). The calculation of chlorophyll content (mg/L) was determined using the formulas [1-3].

$$\text{Chlorophyll A} = 1.07\ (\text{OD } 663) - 0.094\ (\text{OD } 645) \quad [1]$$

$$\text{Chlorophyll B} = 1.77\ (\text{OD } 645) - 0.28\ (\text{OD } 663) \quad [2]$$

$$\text{Total chlorophyll} = 0.79\ (\text{OD } 663) + 1.076\ (\text{OD } 645) \quad [3]$$

Formulation and evaluation of tablet from *S. androgynus* chlorophyll

S. androgynus chlorophyll tablets were formulated using the direct compression method. All the materials were mixed and stirred until evenly dispersed, then tablets were created using a tablet press machine (rotary tablet press ZP-9B, Develop MC, China). The dose of *S. androgynus* chlorophyll was converted from the effective dose of antianaemia in rats of 0.016 mg/200 g BW (Suparmi et al., 2016b) to the human dose of 0.896 mg. A simplex lattice design (SLD) was adopted to optimize the formulation variables. This design evaluated two factors, the matrix-forming

Table 1. Tablet formulation design.

Ingredient	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)
Magnesium stearate/X1 (mg)	13.15 (2.6)	25.05 (5.0)	1.30 (0.3)	1.30 (0.3)	19.10 (3.8)	13.15 (3.6)	7.25 (1.5)	25.05 (5.0)
MCC (Avicel PH 101)/X2 (mg)	275.05 (55.0)	100.05 (20.0)	450.05 (90.0)	450.05 (90.0)	187.55 (37.5)	275.05 (55.0)	362.55 (72.5)	100.05 (20.0)
S. androgynus chlorophyll (mg)	0.896 (0.2)	0.896 (0.2)	0.896 (0.2)	0.896 (0.2)	0.896 (0.2)	0.896 (0.2)	0.896 (0.2)	0.896 (0.2)
Talc (mg)	25.05 (5.0)	25.05 (5.0)	25.05 (5.0)	25.05 (5.0)	25.05 (5.0)	25.05 (5.0)	25.05 (5.0)	25.05 (5.0)
Lactose (mg)	185.85 (37.2)	348.95 (69.8)	22.70 (4.5)	22.70 (4.5)	267.40 (53.5)	185.8 (37.2)	104.25 (20.9)	348.95 (69.8)

agent weights of magnesium stearate (X1) and microcrystalline cellulose (MCC) with the brand of avicel PH 101 (X2) were selected as independent variables (Table 1). The physical characteristics of tablets, including size uniformity, weight uniformity, hardness, friability, and disintegration time, were taken as response variables.

Physical and organoleptic analysis of tablet from *S. androgynus* chlorophyll

The physical characteristic analysis of its tablets refers to methods by Legowo et al. (2023). The standard for the physical characteristics of the resulting tablet is the Pharmacope Indonesia 5th edition from the Ministry of Health, Indonesia (Kemenkes-RI, 2013).

Thickness

The uniformity of the thickness of chlorophyll tablets was assessed using a vernier caliper 500 (Kenmaster, China). The tablet thickness must be at most 3 times the thickness and should be at least 4/3 times the thickness.

Weight uniformity

Twenty chlorophyll tablets were weighed alternately using an analytical balance, Henher-BL-H2. The weighing results determined the standard deviation.

Hardness

The hardness of chlorophyll tablets was assessed by exerting pressure until the tablet fractured or broke. The measurement was conducted using a hardness tester (Copley TBF-1000).

Friability

Friability was tested using a friability tester (Guoming CS-2). Eight chlorophyll tablet samples were put into a plastic tool that rotated at 25 rpm. Tests were conducted to see the effect of scratches and shocks on the quality of the tablet using the formula [4].

$$\%\ friability = \frac{Initial\ weight - Weight\ after\ friability}{Weight\ after\ friability} \times 100\% \quad [4]$$

Disintegration time

Six tablet samples were evaluated for disintegration time using a BJ-2 disintegration tester (Guoming, China). A stopwatch measures time.

Statistical analysis

The data of chlorophyll content, characteristics of the dried extracts, and physical properties of the optimum tablet formula were assessed using their arithmetic mean and variability measured by the standard deviation (SD), together with the number of observations (n). The t-student statistical test was employed to discern differences at a 95% confidence level ($\alpha = 0.05$). Design-Expert version 1.1. was used for prediction and SPSS for Windows 24.0 for data analysis.

RESULTS AND DISCUSSION

The present study evaluated the effect of magnesium stearate and microcrystalline cellulose (avicel PH 101) concentration for the optimum tablet formula from *S. androgynus* leaves. In this study, water was used as a solvent to extract chlorophyll from the leaves of *S. androgynus*. This approach aligns with traditional Indonesian methods for preparing natural chlorophyll metabolites, which are abundant in various leafy vegetables. Water is also a halal solvent to

be applied in the tablet formulation for an antianaemia supplement for chlorophyll. Chlorophyll macerated by water effectively resulted in 40% dried extract from 100 g of *S. androgynus* leaves. Table 2 depicts the chlorophyll B concentration was higher than chlorophyll A concentration, with a total chlorophyll of 10.68 ± 0.02 mg/L. The percentage of moisture content, drying sequence, solubility in water, and solubility in ethanol have met the quality standards of *S. androgynus* leaf extract used for health, according to *Farmakope Herbal Indonesia* from the Indonesian Ministry of Health (Kemenkes-RI, 2017).

The health effects of natural chlorophyll metabolites are influenced by their abundant presence in certain plants, broad occurrence in nature, and their breakdown during food preparation and human digestion (Hosikian et al., 2010). Chlorophyll possesses a chemical composition that closely resembles that of hemoglobin. Due to this resemblance, it is anticipated that tissue regeneration will be enhanced comparably by promoting a rapid interchange of carbon dioxide and oxygen (Hosikian et al., 2010). Natural chlorophyll was reported to have an antioxidant activity based on protecting linoleic acid against oxidation and/or preventing the decomposition of hydroperoxides instead of based on the ability to donate hydrogen (Hosikian et al., 2010). Thus, it can be a potential active pharmaceutical ingredient (API) for curing iron deficiency anaemia. In our previous research, the chlorophyll extract of *S. androgynus* leaves has shown a curative effect in atrium nitrite-induced anaemia mice and rats (Suparmi et al., 2016a; 2016b; 2021). Due to the instability of the chlorophyll extract of *S. androgynus* leaves concerning this study's temperature, light, pH, and oxidation, we formulated the extract into the tablet dosage. Microcrystalline cellulose (MCC) is an excipient in the tablet formulation, which enhances flowability and minimizes moisture absorption. These excipients can preserve a stable formulation and improve processing without degrading the bioactive ingredients in the extract (Zhao et al., 2022).

Table 3 shows the effect of the following physical properties: Thickness, weight, hardness, friability, and disintegration time of the tablets prepared by different Mg stearate and MCC concentrations. All the physical characteristics of the tablets in 8 formulations created by SLD were found to be within the specified range (IP Limit—no less than 95% and no more than 105%). Using the Design-Expert software, SLD can evaluate the influence of formulation variables for each preparation using different methods. Design-Expert can reduce formulation development time and has a high success rate, as evidenced by the degree of desirability and percentage of prediction error (Hidayat et al., 2020).

T-test analysis (Table 4) showed that there is no significant (p>0,05) difference between all physical tests of tablets and SLD predictions. Thus, all the formulas were optimal. The disintegration time for the tablets formulated using Formula 2 (magnesium stearate of 5% and MCC 20%) has the shortest disintegration time among other formulations. The disintegration duration indicates the breakdown and separation time of the tablet into multiple particles within a specific medium (Gupta et al., 2009). The release of active substances from tablets will affect the amount of active substances that are dissolved and absorbed. The faster the disintegration time, the greater the release of the active substance and the quicker it will be absorbed so that the effects of the drug will be felt more quickly to achieve its therapeutic effects (Nicholson, 2009; Perucca, 2009).

Table 2. Chlorophyll content and characteristics of the dried extracts.

Parameter	**Value (mean ± SD)**	**Standard** (Kemenkes-RI, 2017)
Chlorophyll a concentration (mg/L)	0.45 ± 0.00	
Chlorophyll b concentration (mg/L)	0.27 ± 0.00	
Total chlorophyll concentration (mg/L)	0.59 ± 0.00	
Moisture content (%)	5.45 ± 0.36	<10.00%
Drying sequence (%)	6.20 ± 0.08	<10.00%
Solubility in water (%)	38.86 ± 2.65	>4.80%
Solubility in ethanol (%)	59.25 ± 0.11	>6.20%

Data represent mean ± SD (n = 3).

Table 3. Physical properties of optimum tablet formula.

Formulation	Thickness (cm), n = 20	Weight (g), n = 20	Hardness (kg), n = 6	Friability (%), n = 20	Disintegration time (min), n = 6
F1	0.34 ± 0.0	0.50 ± 0.0	5.73 ± 3.07	0.34	30.19
F2	0.32 ± 0.0	0.49 ± 0.0	5.22 ± 2.01	0.30	5.19
F3	0.32 ± 0.0	0.49 ± 0.0	6.17 ± 3.22	0.09	219.46
F4	0.33 ± 0.0	0.49 ± 0.0	8.59 ± 3.82	0.09	242.94
F5	0.33 ± 0.0	0.50 ± 0.0	8.59 ± 2.96	0.84	28.69
F6	0.35 ± 0.0	0.50 ± 0.0	6.60 ± 3.03	0.25	37.19
F7	0.33 ± 0.0	0.49 ± 0.0	11.14 ± 2.97	0.09	47.59
F8	0.33 ± 0.0	0.49 ± 0.0	5.91 ± 2.33	0.20	56.34

Data represent mean ± SD (n = number of tablet samples tested).

Tabel 4. T-test analysis on the difference between all physical tests of tablets from the optimum formula and the SLD prediction.

Parameters	Physical test	SLD prediction	p-value
Size uniformity (cm) n = 20	3.60 ± 0.02	3.72	0.500
Weight uniformity (% RSD) n = 20	1.0 ± 0.15	0.65	0.590
Hardness (kg) n = 6	7.19 ± 0.42	5.57	0.502
Friability (%) n = 20	0.80 ± 0.12	0.27	0.506
Disintegration time (min) n = 6	0.50 ± 0.008	0.51	0.374

CONCLUSION

This study utilized a simplex lattice design (SLD) to predict the ideal concentrations of magnesium stearate and microcrystalline cellulose (MCC) to optimize the physical properties of tablet formulations from *S. androgynus* chlorophyll. The findings indicate that the concentrations of these excipients significantly influence key tablet characteristics, including thickness, weight, hardness, friability, and disintegration time. Among the formulations, Formula 2, containing 5% magnesium stearate and 20% MCC, demonstrated the most favorable disintegration time, suggesting it is the optimal formula for this type of tablet. Further research on the therapeutic benefits and potential toxicities of *S. androgynus* chlorophyll tablets is recommended to explore their role in preventing iron deficiency anaemia.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Suparmi S	Latifah F	Lestanu LA	Fasitasari M	Rustanti N
Concepts or ideas	x				
Design	x				
Definition of intellectual content	x				
Literature search	x		x		
Experimental studies	x	x	x	x	
Data acquisition	x	x	x		
Data analysis	x	x	x		
Statistical analysis	x	x	x		
Manuscript preparation	x				
Manuscript editing	x	x	x	x	x
Manuscript review	x	x	x	x	x

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