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RESEARCH ARTICLE

# Optimisation of lozenge formulation from Citronella (*Cymbopogon nardus* (L.) Rendle) extract with various binding materials using a simplex lattice design method

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## Abstract

**Background:** Antibacterial and antifungal activities of citronella grass extract are widely used, but there is no pleasant dosage form for patients who are unable to swallow. Citronella grass (*Cymbopogon nardus (L.)* Rendle) extract lozenges were prepared with wet granulation method containing natural or synthetic polymer (PGA/PVP/gelatin) binders. **Objective:** This study aimed to determine the most optimal lozenge formula using the simplex lattice design method with Design Expert software 13.0.0. **Methods:** The extract was made using maceration method with 70% ethanol as solvent. Furthermore, the preparation of tablets and physical, chemical and stability tests were perfomed. **Results:** The results of the physical test showed that Formula IV with a ratio of PGA:PVP:Gelatin of 0.5:0.5:0 was optimal with a hardness value of 48.85 ± 0.46 kg/cm$^2$, dissolving time of 684 ± 7 seconds, and friability value of 0.31 ± 0.136%. Chemical testing by the total phenolic content of the extract using the Folin-Ciocalteu method at a wavelength of 750 nm obtained a 78.262 mgGAE/gram extract. Moreover, the optimal tablet obtained a 0.063 ± 0.027 mgGAE/gram extract, and stability testing at room temperature for 28 days found that the obtained lozenges were stable. **Conclusion:** It can be concluded that the combination of PGA (natural polymer) and PVP (synthetic polymer) binders with a ratio of 1:1 can produce optimal and stable citronella grass extract lozenges.

## Introduction

*Cymbopogon sp.* is one of the spice plants that is abundantly available in Indonesia. This plant may be cultivated in the lowlands and highlands up to an altitude of 1,200 meters above sea level (Poerwanto, 2010). In addition, Cymbopogon plants do not require special care; hence, they can be cultivated in the garden. There are two kinds commonly known in Indonesia, namely: lemongrass (*Cymbopogon citratus Stapf*) (Carlini *et al*., 1986) and citronella grass (*Cymbopogon nardus* (L.) Rendle) (De Toledo *et al*., 2016).

Citronella grass (*Cymbopogon nardus* (L.) Rendle) is known to have abundant pharmacological properties due to the presence of antioxidants (Anwar, Ningtiyas & Simanjuntak, 2020), antibacterial, antimalarial, and antifungal properties (Brito *et al*., 2021; Subramanian *et al*., 2015) Antifungal activity is due to the content of secondary metabolites such as tannins, terpenes, alkaloids, flavonoids and phenolics. This compound was found to have antifungal and antibacterial activities against various human pathogens (Wei & Wee, 2013).

A lozenge is a pharmaceutical dosage form that can release the active substance slowly and uniformly in the delivery through the mucous membrane. They treat

local irritation or infection in the oral cavity or throat, such as bad breath and canker sores. Lozenges dissolve or disintegrate in the oral cavity in less than 30 minutes. They are excellent for people who have difficulties swallowing hard pills, such as pediatric and geriatric patients. The hardness of lozenges is higher than regular tablets, which is 7-14 kg (Cooper & Gunn, 1975).

A binder is an additive that is added to provide a cohesive force (integration) to the active pharmaceutical ingredient (API) and other excipients. Therefore, the physiochemistry of the binder impacts the functional properties of a dosage form to form a compact and cohesive solid dosage form (Kestur & Desai, 2016). Determination of the amount of the binder is very critical. Furthermore, when the binders are given in small amounts, they may result in capping, lamination, or chipping. They may increase the hardness of the tablet when administered in large quantities (Baroutaji *et al*., 2017).

Natural binders can be in the form of sugars such as sorbitol, sucrose, Pulvis gummi arabicum (PGA), and glucose. Meanwhile, some are derived from natural polymers such as gelatin, acacia, and starch or semi-synthetic polymers such as polyvinyl pyrrolidone or povidone (PVP) methylcellulose, and ethyl cellulose. In this study, three tablet binders, namely PGA, PVP, and gelatin, were used at different concentrations. This study aims to find innovation in qualified lozenges dosage form from citronella grass extract. It is expected to be applied in the oral cavity and throat and developed into phytopharmaca.

## Methods

### *Material*

Fresh citronella grasses (*Cymbopogon nardus* (L.) Rendle) were obtained in Gunung Putri, West Java, Indonesia. The extraction solvent, ethanol absolute (Merck), was diluted into ethanol 70%, which was an analytical grade. PVA, PVP, and gelatin binding agents were used. Mannitol, lactose, magnesium stearate, and talc were employed as excipients in the lozenges' formulation and were purchased from PT. Dwilab Mandiri Scientific. These excipients were pharmaceutical grade.

### *Extract preparation*

Fresh citronella grass (*Cymbopogon nardus* (L.) Rendle) was rinsed and dried in an oven set at 40°C. In this investigation, only the leaves and stems were used, and a rotary evaporator concentrated the extract after the dried herb was macerated in 70% ethanol for 24 hours (65°C, 60 rpm).

### *Measurement of total Phenolic compound*

The Folin-Ciocalteu method measured the total phenolic compound (Singleton, Orthofer & Lamuela-Raventós, 1999) with adjustments. The technique used 70 nm wavelength and 120 minutes of operating time. Subsequently, the absorbance of a concentration series of gallic acid was measured at the maximum wavelength. The linearity was calculated by determining the correlation between the 50, 75, 100, 125, and 150 ppm concentration series and the absorbance. The Miller method was used to determine the detection and quantitation limits from the linear regression curve. The method's accuracy and precision were also determined using the standard addition method of gallic acid into the tablet matrix. Furthermore, the total phenol content in the extract and the tablet was measured using this method and expressed in mg GAE (gallic acid equivalent)/gram sample.

### *Production of Lozenges*

The lozenges tablet was made using the wet granulation method with 10% Citronella extract as an active compound. The wet granulation method was selected as the manufacturer. The steps were commenced by weighing and mixing the ingredients of the intragranular phase, namely citronella grass extract, mannitol, and lactose, until homogeneous. Manufactured binder solutions from PGA, PVP, and gelatin were under the predetermined concentration ratio. The binder solution was subsequently mixed into the intragranular mixture until a granular mass was formed with a snowball-like consistency. Granulation was carried out using tablet sieve number 230, and the wet granules were dried in an oven at 50°C for ± nine hours until the water content met the requirements of less than 2%. Finally, the dry granules were sieved with a sieve shaker, tested for quality, and compressed into a lozenges dosage form.

The excipients used are 60% mannitol, 1% magnesium stearate, 15% lactose and 9% talc. Seven variations of binding agent formulations of PGA, PVP and gelatin were used as 5% of total materials. The content ratio of PGA: PVP: gelatin in each of the formulas were as follows: formulas I, II, III, IV, V, VI and VII used 1:0:0, 0:1:0, 0:0:1, 0.5:0.5:0, 0:0.5:0.5, 0.5:0:0.5 and 0.34:0.33:0.33 respectively.

### *Quality test*

The quality of the granule was measured from loss of drying, mass flow rates of granules, angle of repose and tap density. The quality of tablets was determined by hardness, friability, friction and disintegration time.

According to United States Pharmacopeia (USP), the parameters were measured using a standard analytical tester.

***Data analysis technique***

The data was analysed using the simplex lattice design method with Design Expert software 13.0.0 (trial). The components for optimisation were the concentration and type of binder, and the optimisation parameters were the hardness, friability and disintegration time. Therefore, the binder's optimal concentration of good lozenges can be determined.

## Results

***Evaluation results***

The superimposed contour lot shows that the optimum binder combination for lozenges was PGA: PVP: gelatin of 0.5:0.5:0 (Figure I).

The granules' evaluation of all formulas shows that Formula IV has met the requirement of flow rate, angle of repose, moisture content, and tap density (Table I).

The physical evaluation was expressed with an equation (Table II). This equation is used for determining the response of the mixture design of three binder materials on hardness, friability and disintegration time.

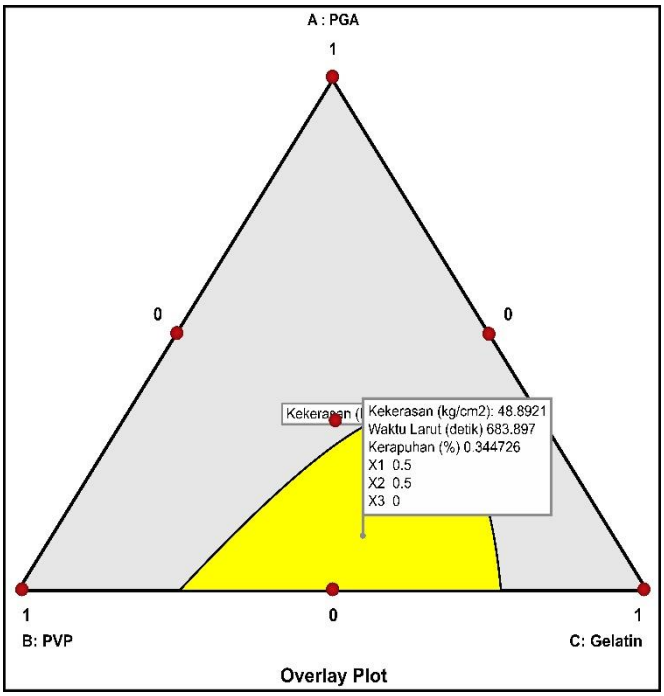


**Figure I: Superimposed Contour Plot (hardness, disintegration time, and friability parameters)**

**Table I: Granule evaluation result for seven formulas**

| Evaluation | Formula | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII |
| **Flow rate (g/s)** | 234.33 ± 38.48 | 213.67 ± 15.01 | 204.33 ± 0.21 | 212.33 ± 11.72 | 318.0 ± 9.00 | 324.67 ± 451 | 197.67 ± 4.93 |
| **Angle of Repose (°)** | 30.31 ± 0.26 | 33.21 ± 2.27 | 33.58 ± 2.51 | 32.99 ± 1.49 | 33.05 ± 0.81 | 34.69 ± 0.05 | 33.24 ± 1.07 |
| **Moisture Content (%)** | 0.05 ± 0.030 | 0.02 ± 0.008 | 0.02 ± 0.008 | 004 ± 0.004 | 0.04 ± 0.004 | 0.09 ± 0.004 | 0.05 ± 0.017 |
| **Tap Density (%)** | 7.58 ± 0.86 | 4.99 ± 0.98 | 4.60 ± 0.73 | 4.42 ± 0.30 | 6.23 ± 0.02 | 3.21 ± 0.01 | 3.06 ± 0.08 |

**Table II: Optimisation equation on physical evaluation of lozenges**

| Response | Equation |
|---|---|
| **Hardness (N/cm²)** | $Y = 39.8A + 32.6B + 42.6C + 50.7AB + 171.6BC + 107.8AC - 180ABC$ |
| **Friability (%)** | $Y = 0.2A + 0.4B + 0.2C - 0.18AB + 0.7BC - 0.1AC - 0.17ABC$ |
| **Disintegration Time (seconds)** | $Y = 499A + 454B + 419C + 830AB + 2116AC + 2154BC - 2380.44ABC$ |

A =PGA (Pulvis Gummi Arabicum), B= PVP (Polyvinylpyrrolidone), C= Gelatin

***Determination of total phenolic content in extract and tablet***

The validated Folin-Ciocalteu method was used to determine the total phenolic content in the extract and tablet. The technique used 750 nm wavelength and 120 minutes of operating time. This method's detection limit was 10.69 µg/mL, the limit of quantification was 32.41 µg/mL, the accuracy was 98 – 102 %, and the RSD value was 0.5% – 0.6%. Since all the parameters met the requirement, this method was used to analyse the total phenolic content of the extract and lozenges

tablet. This method found that the total phenolic content in the lemongrass extract was 78.262% w/w (equivalent to 78.262 mgGAE/g extracts), and the optimum tablet formula showed that the total phenolic content was 0.063% w/w or equivalent to 0.063 mgGAE/g tablets.

## Discussion

### *Preparation of lozenges*

Lozenges are compressed tablets formulation without disintegration. Lozenges are solid dosage forms containing medicinal ingredients and additives intended to dissolve slowly in the oral cavity to provide local effects (Ansel, Allen & Vlachou, 1999). The manufacture of compressed lozenges is the same as making conventional tablet dosage forms.

### *Granule evaluation*

The water content in the granules was measured, and the granules obtained are expected to be stable and easily stored, with good moisture content data in 1-5% (Rowe, 2009). This can affect tablets' hardness, friability and disintegration time, and high water content may cause the granules to not flow appropriately during moulding. Therefore, the granules may stick to the punch and dye and cause capping.

### *Flow rate*

The methods commonly used to test the flow properties of granules are the measurement of the angle of repose, compressibility index or Hausner ratio and flow rate through an orifice and shear cell. Testing the flow rate is conducted by measuring the speed of the granules to flow per unit of time, which affects the length and number. Granules that do not flow well cannot fill the printing space optimally and consistently; hence the resulting lozenges do not have a uniform weight. In this study, the granules produced from the seven formulas showed a phenomenal flow rate of > 10 grams/second. The interpretation is that the granules fill the dye uniformly and consistently, allowing the uniformity of lozenge weight.

### *Angle of repose*

The angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles (United States Pharmacopoeia, 2021). A measure of powder cohesiveness can be seen in the moment of interaction between particles and their gravitational attraction. The angle of repose test results of 31 - 35$^o$ were obtained for Formula I to VI. Therefore, the interpretation of granular flow is reasonable, while the angle of repose of 39.39$^o$ ± 0.10$^o$ for Formula VII is classified as fair-aid without flow property.

### *Compressibility index or Hausner ratio*

This test is a common, simple and fast method for predicting the flow properties of granules. Furthermore, it does not directly measure the bulk density, size and shape, surface area, moisture content and cohesiveness of the material because these can affect the observed compressibility index. This is determined by measuring the powder's bulk and tapped volume. Based on the data obtained for all formulas, the compressibility index is ≤ 10, and the interpretation has a very good flow character.

### *Lozenges evaluation*

Good lozenge strength has a hardness property between 7 - 14 kg. A total of 20 lozenges were evaluated using a 100-rotation friability at 12 rpm. The tested lozenges have a value of < 1% and disintegrate slowly in < 30 minutes.

### *Formula optimisation*

Formula optimisation was carried out to determine the best formula using data from the evaluation results of the manufactured dosage or to obtain the combination of a product or the material from several available options. The determination from the composition of three different binders, namely PGA, PVP and gelatin, was processed by Design Expert software 13.0.0 (trial) with the Simplex Lattice Design method. Furthermore, the optimum formula result is Formula IV with a combination PGA:PVP: gelatin binder at a concentration ratio of 0.5:0.5:0. Simplex Lattice Design is a mixture optimisation method with a proportion between 0 (0%) and 1 (100%). Formula IV has a degree of desirability close to 1, and the evaluation data on lozenges of citronella grass extract obtained from the seven formulas were entered into Design Expert software. The combination of binders can increase hardness and dissolution time and reduce friability; hence, an optimum binder ratio is needed. PGA as a binder can be used at concentrations of 1 - 5%, PVP 0.5-5% (Rowe, 2009), and gelatin 5 - 10%. The concentration affects the quality of the resulting lozenges. In addition, VP and gelatin can produce soft and hard lozenges with fast and slow disintegration times. PGA can increase the viscosity due to forming more compact granule bonds and cause hard tablets and long disintegration times. Therefore, the combination of the three binder materials should be examined using the Simplex Lattice Design approach by

evaluating the hardness, friability, and disintegration time. Responses were recorded in terms of hardness (7 - 14 kg or 68,649 - 117,684 N/cm$^2$), friability (< 1%), and dissolution time (< 30 minutes).

Based on the superimposed results, the presence of a combination of binders can increase hardness and disintegration time as well as reduce friability. Therefore, an optimum binder ratio is needed. Formula IV shows the optimum ratio, which is supported by the granule test.

***Stability test***

A stability test was carried out on all seven formulas, starting from day 0 to 28 at room temperature. Tablets were stored in an airtight container, covered with aluminium foil, and inserted in the silica gel. The test result data showed that the lozenges of citronella grass extract with Formula IV are stable during 28 days of storage.

***The total Phenolic content***

The antimicrobial activity of Citronella mainly comes from its phenolic compounds. Therefore, the phenolic content was determined as an internal characteristic of the lozenges. Since the phenolic content of the extract and the tablets differs significantly, another method, such as microdilution, is needed to measure antimicrobial activity.

## Conclusion

A combination of PGA, PVP, and gelatin as a binder solution with a concentration of 1:1:0 may produce lozenges that meet the requirements and are stable for 28 days of storage at room temperature.

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