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# Shelf life quality of *Labisia pumila* extract and effect of granulation on dissolution rate

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**Abstract:** Lyophilized from plants extract is a process to prolong the amounts of phytochemicals in a solid form. The bioactive compound in LP, which is Gallic acid should be stable and bioavailable during the downstream processing such as drying process in order to achieve its optimum health benefits. Therefore, this study focuses on the impact of lyophilize methods such as spray drying followed by granulator method in order to determine the stability of bioactive compound in LP during drying and storage. After the powdered produce, it will test on the model of human digestion (dissolution study) either the amounts of phytochemicals still remain stable. Finally, shelf-life quality analysis and the dissolution rate (capsule size 0 and 1) were carried out. The results obtained show the methods employed in this study can be used for analysis of quality content and dissolution tests of LP capsules. For LB in shelf-life quality study, parameters for appearance and TYMC showed no significant difference after 3 months. For pH, the slight decrease in pH level will increase the number of TAMC in powder form. Compared to granule form, the pH level more consistent and the number of TAMC also in a control condition. The dissolution of capsules containing powder and granulated of LP showed a high percentage of bioactive compound released into the dissolution medium, although the bioactive compound content of herbal products (capsule size 1) was found to be half that of capsules size 0. Through observation for both capsules size content of LP, the maximum dissolution was achieved after 100 min.

**Keywords:** *Shelf life quality, Granulation, Dissolution time, Labisia pumila and Gallic acid.*

## INTRODUCTION

Lyophilized from plants extract is a process to prolong the amounts of active ingredients or phytochemicals in a solid form. This lyophilized process will focus on spray drying, freeze-drying and granulation. Plants mainly consist bioactive compound that responsible for health beneficial effects. However, the good bioactive active components have to be protected from environment delay degradation and stabilize them during formulation and storage. In addition, plant extract in the powder form offering wider application in the product formulation compared to the liquid form. The spray drying yields a fine powder with generally poor handling properties such as poor aqueous solubility, wettability, sink ability, therefore often followed by agglomeration process to tackle this problem because this process is able to form higher particle size and porous morphology which are desired properties in terms of high-quality powder.

*Labisia pumila* (LB) has been extensively used to assist childbirth and during the postpartum period [1]. Other studies show that this plant has anti-oxidant [2] and anti-inflammatory properties due to its presence of phenolic compound [3]. Moreover, this plant able to exert uterotrophic effect and regulates body weight gain by

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modulating the secretion of adipocytes in adipose tissues [4]. The bioactive compound in LP should be stable and bioavailable during the downstream processing such as drying process in order to achieve its optimum health benefits. Therefore, this study focuses on the impact of lyophilized methods such as spray drying followed by granulator method in order to determine the stability of bioactive compound in LP during drying and storage. After the powdered produce, it will test on the model of human digestion (dissolution study) either the amounts of active ingredient or phytochemicals still remain stable.

## MATERIALS AND METHOD

### A. Powder and granulation process

Extraction ratio 1:10 of LP in 4 hours with extraction temperature 105-112$^0$C. Spray Drying (Niro Spray Dryer, Gea, Germany) at 180$^0$C with spraying speed 30L/hours. Strea-1, Gea Nyro from Germany were used for the granulation process in 30 minutes. Monohydrate as main material added with Polyvinyl povidone (PVP) K30 as a binder coating with LP powder to produce granulation form. The final size of granules is in between 100-150μm. Powder and granules of LP, were pack 500 mg in capsules size 0 and 1. Finally, the dissolution rate and shelf life quality analysis were carried out [5].

### B. Shelf life quality analysis

1. Appearance

The appearance of the LP extract powder was analyzed using observation in 3 months. The colour and its changes were recorded [6].

2. pH

Weight 20 g powder and granule of LP in a plastic bottle and add 50 ml distilled water. Shake for a while and leave it standing overnight (more than 16 hours). Calibrate the pH meter using buffer pH 4.00 and pH 7.00. Shake the sample and read the pH value [6].

3. Sterilization

Sterilizer was first cleaned with cotton soaked in 95% ethanol to ensure microbe-free sterilizing environment. Petri plates and pipette were washed with distilled water and dried. Petri plates were then placed in an oven (Memmert, Germany) at 180$^0$C for 2 hr [7].

4. Serial dilution

The serial dilution technique was employed according to the work by [8][9] with slight modifications. For the observation of mould and yeast, 1 ml of bakery product was transferred to 100 ml of sterilized distilled water. It gave dilution of 1:10. Briefly, 1 ml of suspension from 1:10 was transferred to second test tube which gave 1:100 dilutions. Similarly 1:1,000, 1:10,000, and 1:100,000 dilutions were made. Dilutions were transferred to the sterilized Petri plates containing media. Then these Petri plates were incubated at 30 $\pm$ 2$^0$C for 3–7 days for microscopic and macroscopic identification.

5. Total aerobic microbial count (TAMC), Coliform, *Salmonella* and *Escherichia coli*

Enumeration of TAMC was done using nutrient agar (NA). Eosin methylene blue (EMB) agar was used for the coliform count, *Salmonella* agar was used for *Salmonella* count, MacConkey Agar (MAC) was used for *Escherichia coli*. All cultures were incubated in duplicate at 37$^0$C for 24 hours except for coliform organism(s) which were incubated at 37$^0$C and 44$^0$C for 24 hours. All media used were prepared according to the manufacturers' instructions.

6. Total yeast and mould count (TYMC)

Potato dextrose agar was prepared by weighing 4 g of potato dextrose agar and poured it into a beaker. About 100 ml of water was added and heated for 2–3 min. Continuous stirring was provided to the solution during heating to make homogenous mixture. Malt extract agar was prepared by dissolving 2 g of malt extract and 2 g of agar in distilled water to make the volume up to 100 ml. The solution was mixed and gently heated till boiling. After preparation of saturated solutions, 10 ml of the solution was poured in three test tubes. The test

tubes were then covered tightly with cotton plugs. This medium was then autoclaved at about 15 lbs and $121^0$C for 15 min. After autoclave, media from test tubes were poured into freshly sterilized Petri plates and set to cool. After incubation plates, numbers of Petri plates were counted and multiplied by dilution factor to find out the number of spores per gram of a sample [8][9].

No. of spores/g = No. of colonies × Dilution factor (1)
Dilution factor = Reciprocal of dilution (e.g., $10^{-5} = 10^5$).

i. Identification

Fungi were identified on the basis of morphological and cultural characteristics such as the colour of the colony, surface, appearance according to the methods described in [10].

ii. Percentage contribution of each species

To find out percentage contribution following formula was used:

$$\% \text{ Contribution} = \frac{\text{Total no. of CFU of an individual species}}{\text{total number of CFU}} \times 100 \quad (2)$$

• CFU: colony-forming unit

### C. Dissolution Rate Analysis

The dissolution rate of LP for granulated and non-granulated samples at different concentration was studied in 0.1N hydrochloric acid (900 mL) using USP Type 2 (M/S Labindia DS 8000) dissolution rate apparatus (Chowdry *et. al.*, 2011). The paddle stirrer was set to 50 rpm with the depth of 25mm at 37 ± $1^0$C. 5mL samples were taken out from each tube for 10 minutes intervals using 0.45µm filter and analysed using HPLC. The sample withdrawn was replaced with the fresh solution at each time of sampling. The cumulative percentage of bioactive compound from in vitro dissolution testing is shown as in Equation (1-3). All experiments were conducted in triplicate [11].

Concentration of bioactive compound (µg/ml) = (slope x absorbance)) ± intercept (3)

$$\text{Amount of bioactive released mg/ml} = \frac{\text{Concentration x Dissolution bath volume x Dilution factor}}{1000} \quad (4)$$

$$\text{Cumulative percentage release (\%)} = \frac{\text{Volume of sample withdrawn (ml)}}{\text{Bath volume (V)}} \times P(t-1) + Pt \quad (5)$$

Where,

Pt = Percentage release at time t
P(t-1) = Percentage release previous to 't'

## RESULT AND DISCUSSION

For LB extract (powder form) in shelf-life quality study, parameters for appearance, TYMC, Coliform, *Salmonella* and *Escherichia coli* showed no significant difference after 3 months' time duration. For pH, related to the number of TAMC there are significant different results. In powder form (Figure 1), the decrease of pH from 5.50 to 5.20 will affect the number of TAMC in the powder form. The number of TAMC increased from 1.5 x $10^3$ CFU/g to 2.5 x $10^3$ CFU/g simultaneously by the pH decrease. The number of TAMC increased because at the lower pH, in general the aerobic microbes will be more suitable and comfortable to survive [12].

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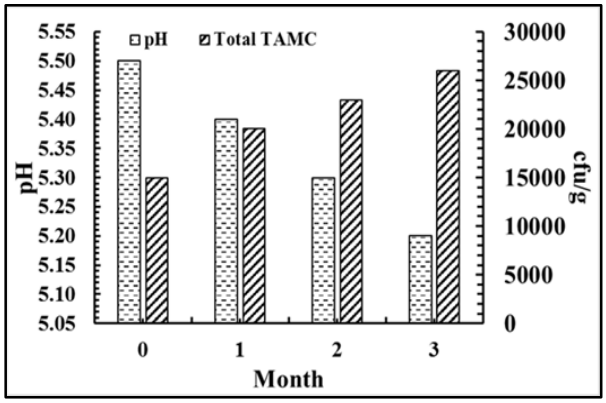


Figure 1. *Labisia pumila* shelf life (Powder)

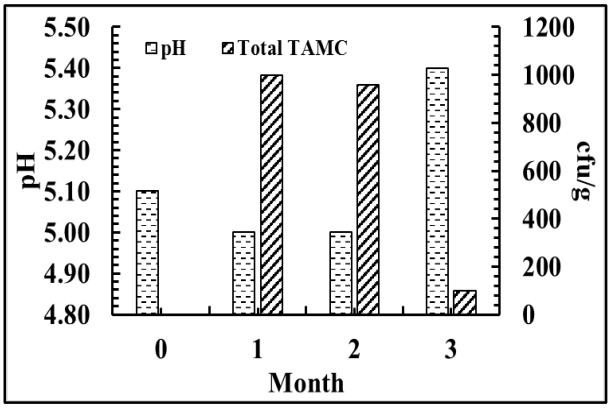


Figure 2. *Labisia pumila* shelf life (Granule)

For LB shelf life in granular form (Figure 2), pH between 5.10 to 5.00, in two months' time duration, shows that the number of TAMC also consistency in between 900 to 1000 CFU/g. Start from month 3, the pH increased rapidly from 5.00 to 5.40 which affected the decreased of TAMC from 900 to 100 CFU/g. This significant result explains according the TAMC which is under pH 5.0 is hard to survive as reported by [13].

Figure 3 and 4 shows the dissolution profile of the capsule containing LP extract powder (500mg) in size 0 and 1. These dissolution results have shown that the bioactive compound which is Gallic alcid simultaneously absorb into the water-based solutions. Capsule size 1 was selected for better application base on the earlier dissolution which is 10 minutes faster than size 0. The main reason for size 1 dissolution faster than size 0 is because the surface area is higher than size 0. The significant area of surface which contribute to the faster dissolution was supported by [14]. Both sizes will achieve maximum dissolution rate after 100 minutes of application.

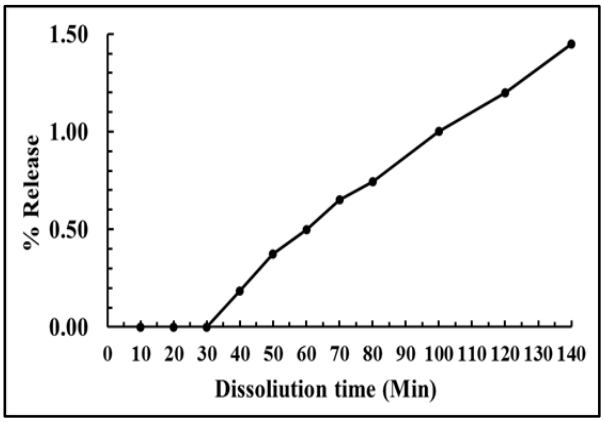


Figure 3. The dissolution profile of capsule containing LP extract powder (500mg) with capsule size 0

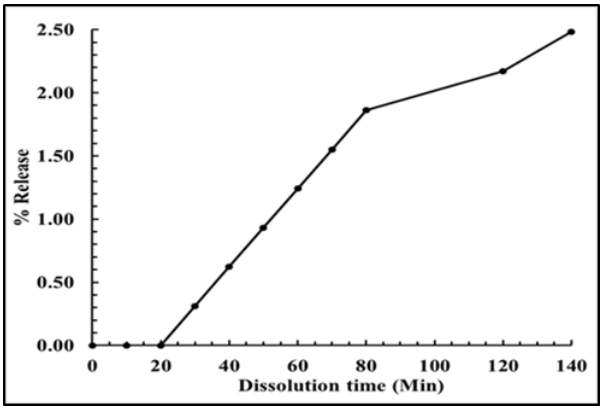


Figure 4. The dissolution profile of capsule containing LP extract powder (500mg) with capsule size 1

For dissolution profile of capsule containing LP extract granule (500mg) in size 0 and 1 shown in Figure 5 and 6. Capsule size 1 was selected for better application base on the earlier dissolution which is 10 minutes faster than size 0. Similar reason as explain before, surface area is the main factor for the dissolution rate [15]. For size 1, after 20 minutes application time, the % of bioactive compound release will consistent with time compared to size 0, where the % release will increase from time to time.

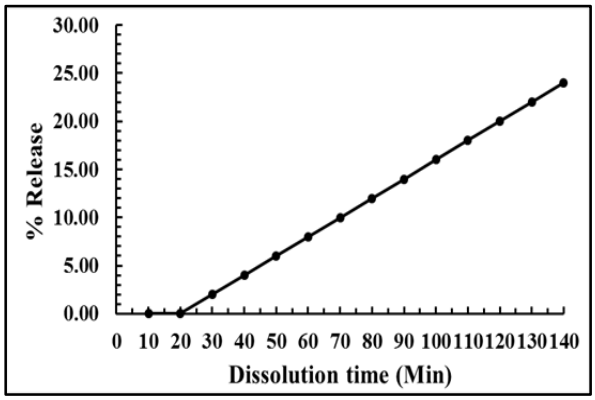


Figure 5. The dissolution profile of capsule containing LP granulated extract powder (500mg) with capsule size 0

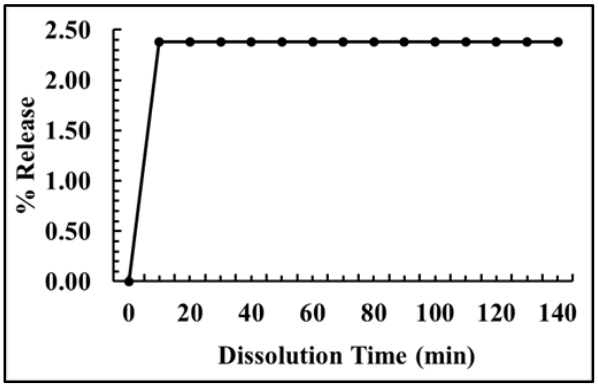


Figure 6. The dissolution profile of capsule containing LP granulated extract powder (500mg) with capsule size 1

## CONCLUSION

The results obtained allow us to suggest the methods employed in this study can be used in the quality control (analysis of quality content and dissolution tests) of LP capsules since the methods were demonstrating selectivity, linearity and range, precision (repeatability and intermediate precision), accuracy and robustness. For pH, the slight decrease in pH level will increase the number of TAMC in powder form. Compared to granule form, the pH level more consistent and the number of TAMC also in a control condition. The dissolution of capsules containing powder and granulated of LP showed a high percentage of bioactive compound released into the dissolution medium, although the bioactive compound from LB (capsule size 1) was found to be half that of capsules size 0. Through observation for both capsules size content of LP, the maximum dissolution was achieved after 100 min.

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