

Physicochemical Stability Studies of Tablet Containing A Mixture of *Sonchus Arvensis* L Leaves Extract and *Lumbricus rubellus* Powder

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ABSTRACT

The aim of this study was to determine the physical and chemical stabilities of tablets containing *Sonchus arvensis* L leaves extract and *Lumbricus rubellus*. The tablets were produced by wet granulation process. The stability test was observed during a 3-month accelerated stability under $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH storage conditions. The tablets were evaluated every month for the physical properties include organoleptic, weight variation, friability, hardness, and disintegration time, while the chemical properties of the tablets include Rf value, and spotting profile was examined using TLC-Densitometry. The result of the physical stability test up to the third month showed that the tablets of *Sonchus arvensis* leaves extract and *Lumbricus rubellus* met the requirements of Indonesian Pharmacopeia 5th and United State Pharmacopeia 32. The qualitative chemical property stability test on the tablets also demonstrated that luteolin as a marker compound remained stable in the tablets after three months of storage in an accelerated condition. Conclusion, the formulation of a tablet containing *Sonchus arvensis* leaves extract and *Lumbricus rubellus* stable after this stability studies.

Keywords: Stability, tablet, *Sonchus arvensis*, *Lumbricus rubellus*.

INTRODUCTION

Atherosclerosis, one of the chronic diseases, occurs when the blood vessel walls are damaged due to inflammation and fat plaque formation¹. Several types of medicine have been used for atherosclerosis treatment, such as urokinase, streptokinase, staphylokinase, and recombinant pro-urokinase, but these medications have many side effects including bleeding, hypotension, nausea, and vomiting^{2,3}. In addition, atherosclerosis treatment using those drugs is somewhat costly⁴. Therefore, the formulation of safer, more affordable anti-atherosclerosis drug can be one of the solutions. Among the many efforts to discover new atherosclerosis therapeutic agents is the use of natural resources found in *Sonchus arvensis* L and *Lumbricus rubellus*.

One of the benefits of *Sonchus arvensis* is a diuretic. It has been reported that *Sonchus arvensis* leaves extract with the dosage of 514 mg/kg BW for a human had a relatively high diuretic effect⁵. Furthermore, the luteolin compound in *Sonchus arvensis* leaves showed an anti-inflammation activity by inhibiting the synthesis of IL-6 and COX-2 to reduce inflammation on blood vessel walls⁶. Meanwhile, *Lumbricus rubellus* contain such proteases as lumbrokinase, earthworm plasminogen activator, and active glycoprotein complex that can function as fibrinolytic or plaque hydrolysis agent in blood vessels³. The extract of earthworms with a dose of 600 mg/kg BW for a human could give a sound thrombolytic effect⁷.

The combination of *Sonchus arvensis* leaves extract, and *Sonchus arvensis* also provides a little fibrinolytic activity. A study reported that the combination of 1 mg of

earthworms and 1.5 mg of *Sonchus arvensis* leaves extract had 18 units/mg of fibrinolytic activity in vitro. This combination was estimated to have a synergistic effect in which the fibrinolytic effect of earthworm extract can hydrolyze plaque while the diuretic effect of *Sonchus arvensis* leaves extract can accelerate plaque removal⁸.

The development of *Sonchus arvensis* leaves extract, and *Lumbricus rubellus* combination as tablets becomes one of the solutions to enhancing the treatment effectiveness of anti-atherosclerosis and increasing the convenience of use. Tablet formulation of *Sonchus arvensis* leaves extract, and *Lumbricus rubellus* was reported to have decent physical characteristics and fulfil the requirements of Pharmacopeia^{9,10}. However, the physical and chemical stability of the tablets in the report mentioned above was unknown.

Based on the explanation as stated earlier, it is deemed necessary to study the physicochemical stability of *Sonchus arvensis* L. leaves extract and *Lumbricus rubellus* combination to find the physically and chemically stable formulation.

MATERIALS AND METHODS

Materials

Sonchus arvensis L, *Lumbricus rubellus* powder, and ethanol were purchased from local suppliers. Starch, lactose, polyvinylpyrrolidone (PVP K-30), Primojel, magnesium stearate, and talcum were obtained from Brataco Ltd. Company Indonesia. All other chemicals used were a reagent of pharmaceutical grade.

Methods

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Table 1: Tablet formulation containing *Sonchus arvensis* leaves extract and *Lumbricus rubellus* powder combination (for one tablet).

Materials	Quantity (mg)
<i>Sonchus arvensis</i> extract	262.5
<i>Lumbricus rubellus</i> powder	200
Lactose	128.89
PVP K-30	3.33
Primogel	52
Magnesium stearate	6.5
Total	650

Preparation of *Sonchus arvensis* leaves extract

Sonchus arvensis leaves extract was obtained through a maceration method followed by evaporation of the solvent using a rotary evaporator (Buchi R-100). The viscous extract of *Sonchus arvensis* was then evaluated through loss on drying test and compound identification. The process to identify the compound of sample extract employed TLC (Camag) method through the cellulose-based stationary phase and mixed mobile phase of butanol: acetic acid: water (4:1:5). The process also used 5% AlCl_3 as a spray reagent. The TLC result was observed under UV light at 254 nm and 366 nm followed by a test to examine the R_f value using a densitometer. The composition of tablet formulations is shown in Table 1.

Preparation of Tablets

The active substances - *Sonchus arvensis* leaves extract and *Lumbricus rubellus* powder - were prepared into tablets using the wet granulation method. The extract and powder, lactose, and primogel were dry-mixed with the binder solution containing PVP. Next, they were added to obtain a damp coherent mass, which was then filtered using a 12-mesh sieve and dried. This dried granular mass was seeped through a 14-mesh sieve to obtain uniform sized granules. Different batches of the granules were then mixed with calculated equal quantities of magnesium stearate and compressed into tablets under a constant pressure using a tableting machine¹¹ (Korsh EK 0).

Evaluation of Tablets

The prepared tablets were evaluated for their active substance, physical appearance, weight variation, hardness, friability, and disintegration time. The flavonoid (active substance) contained in the tablets was assessed using TLC (Camag). The USP weight variation test was performed by weighing 20 tablets individually, calculating the average weight, and comparing the individual weights to the average. The hardness of 10 tablets was determined using the Erweka hardness tester. The friability was

determined by weighing ten tablets after dusting and placing them in a friability tester (Erweka TA 200), which was rotated for 4 minutes at 25 rpm. After dusting, the total remaining mass was recorded, and the friability percentage was calculated. Then, the disintegration time of six tablets per formulation was individually determined in a disintegration tester (Erweka ZT 502) containing aquadest at 37 ± 0.5 °C. The mean disintegration time was later calculated.

Stability Test

The chemical property test was conducted to gain the R_f value and spotting profile using TLC-Densitometry. The stability test lasted for three months in an accelerated condition ($40^\circ\text{C} \pm 2^\circ\text{C}$ and $75\% \text{RH} \pm 5\% \text{RH}$), and the physicochemical analysis of the tablets was performed every month. The data were analysed by comparing the results with the requirements of Pharmacopeia.

RESULTS AND DISCUSSION

Extraction Yield

The result of *Sonchus arvensis* leaves extraction yield was 11.7%. This data has fulfilled the requirement for *Sonchus arvensis* leaves viscous extract that should be no less than 7.5%¹¹.

Loss on Drying Test

The average loss on drying (LOD) of *Sonchus arvensis* leaves viscous extract was 8.47%. Therefore, the extract has met the standard of Indonesian Herbal Pharmacopeia that set the value of LOD at no less than 10%¹¹. It also means that the extract has fulfilled one of the non-specific parameters for determining the standardised quality.

Compound Identification in the Extract

The examination towards the TLC result of *Sonchus arvensis* leaves extract using AlCl_3 spray reagent showed that there were spots of pale yellow under visible light and light purple under 366 nm UV light. The use of AlCl_3 as spray reagent was intended to observe flavonoid compound because AlCl_3 can specifically bind with flavonoid compound and develop yellow color¹². TLC identification test was followed by qualitative TLC-Densitometry that resulted in the identification presented in Figure 1.

Based on the compound identification for *Sonchus arvensis* leaves viscous extract using densitometer, it was predicted that there was a luteolin compound (Substance 2; Figure 1.B; R_f : 0.84) because the R_f value was close to the luteolin standard R_f (Substance 2; Figure 1.A; R_f : 0.86). The difference in R_f value between the sample *Sonchus arvensis* leaves viscous extract, and luteolin

Table 2: Physical evaluation of tablets containing *Sonchus arvensis* leaves extract and *Lumbricus rubellus* powder.

Evaluation	Storage time	Month-0	Month-1	Month-2	Month-3
		\bar{X}	\bar{X}	\bar{X}	\bar{X}
Weight variation	\bar{X}	651.20±1.65	650.60±3.27	651.00±1.76	650.10±2.51
	CV (%)	0.25	0.50	0.27	0.39
Friability (%)		0.097±0.017	0.131±0.032	0.125±0.067	0.334±0.024
Hardness (kgf)		5.44±0.41	5.45±0.76	5.10±0.09	4.91±0.08
Disintegration time (min)		8.18±0.11	10.11±0.01	10.41±0.01	13.24±0.06

Note: CV = Coefficient Variation ; \bar{X} = Average (n=10)

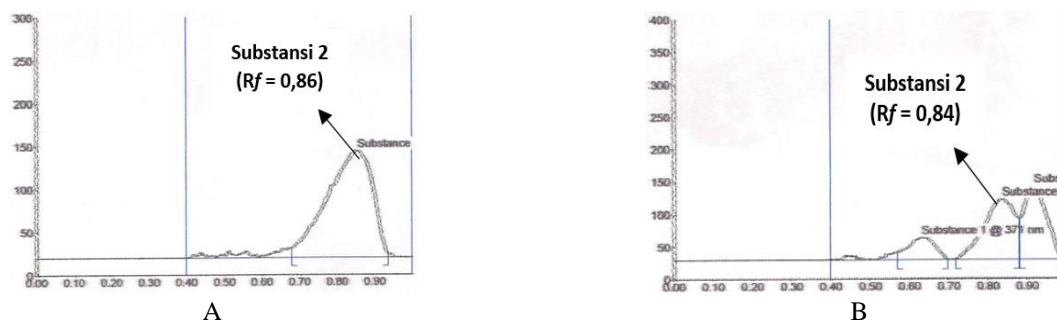


Figure 1: Chromatogram for identification of the sample of *Sonchus arvensis* leaves extract (A) and luteolin as a standard (B).

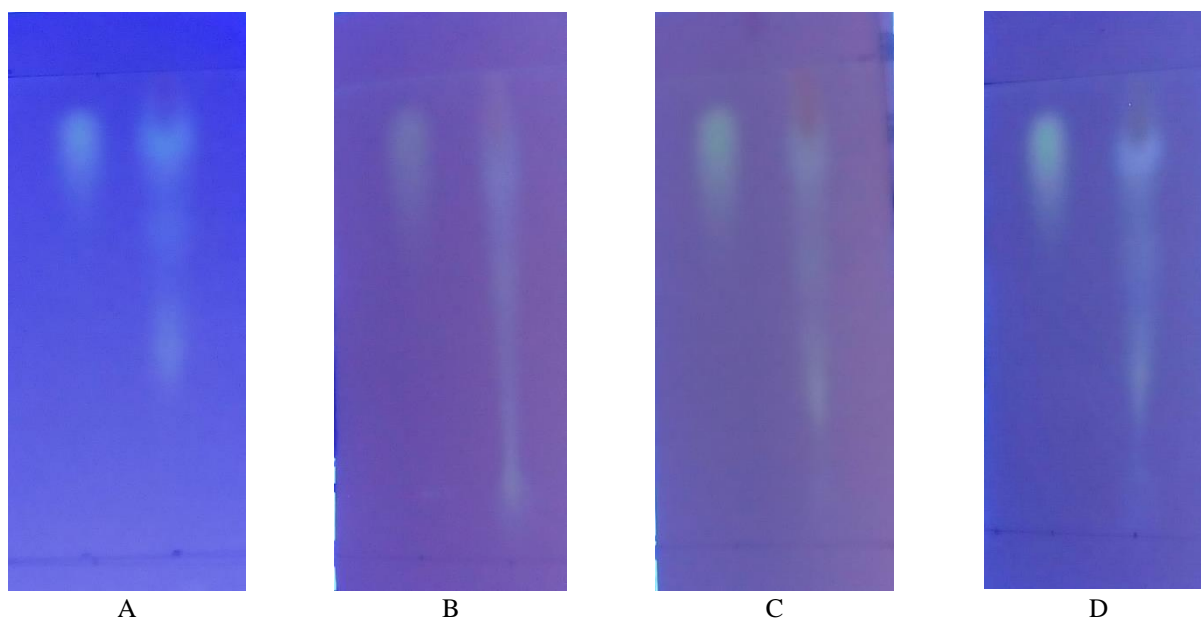


Figure 2: TLC test of the tablet after storage in stability study for 0 month (A), 1st month (B), 2nd month (C), and 3rd month (D).

Table 3: Chemical stability test of the tablets stored in accelerated condition.

Parameter	Storage Period			
	Month 0	Month 1	Month 2	Month 3
<i>R_f</i> Value	Luteolin: 0.88 Tablets: 0.88	Luteolin: 0.88 Tablets: 0.89	Luteolin: 0.88 Tablets: 0.84	Luteolin: 0.84 Tablets: 0.85

standard might be caused by the interaction between luteolin compound and other compounds in the viscous extract that established hydrogen bond or covalent compounds¹³. The relationships could influence the polarity of luteolin compound and affect the *R_f* value of luteolin in the sample viscous extract.

Physical Stability Test for the Tablets

The tablets were tested for their physical stability including the organoleptic properties, weight variation, hardness, friability, and disintegration time. The result of physical evaluation for the tablets is illustrated in Table 2.

The finding showed that the organoleptic properties experienced a change in the third month. A damp, warm storage was predicted as causing the enzyme in the tablets to degrade. Meanwhile, the weight variation in the third month did not change significantly ($p > 0.05$) and remained in the acceptable range ($CV < 2\%$). However, the friability,

hardness, and disintegration time became significantly different in the third month ($p < 0.05$) although theoretically the values of friability, hardness and disintegration time were still in the acceptable range required by Indonesian Pharmacopeia 5 and USP 32^{15,16}.

Chemical Stability Test for the Tablets

The chemical stability of the tablets was examined by observing the value of *R_f* and spotting profile using TLC-Densitometry. The result of a chemical properties stability test of the tablets can be seen in Figure 2 and Table 3.

Table 3 showed that the value of *R_f* in the tablet sample in each month was close to the value of *R_f* in the luteolin standard. The spotting profile was found that additional substances were detected after an accelerated storage. This data was suspected as other compounds that were unravelled and read at a wave of 371 nm as tablet excipient, the complex of the enzyme in earthworms, and

other flavonoid compounds. According to the result of chemical properties evaluation towards the combination of *Sonchus arvensis* leaves extract and *Lumbricus rubellus* tablets, it can be concluded that qualitatively the luteolin compound as the marker compound was estimated to remain stable during the 3-month storage in accelerated condition.

CONCLUSION

The physicochemical properties of the tablets remain stable in an accelerated condition ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \text{RH} \pm 5\% \text{RH}$) for three months. Physically, the weight variation remained the same and met the requirements. Statistically, however, there was a change in the friability, hardness and disintegration time of the tablets although theoretically they still fulfilled the requirements. Meanwhile, the chemical properties of the tablets showed that the luteolin compound in the sample tablet in month 0 up to the 3rd month has the same Rf value as that of the luteolin standard in the densitometry analysis.

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