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Liquid Herbal Mixed of Indian Borage Extract and Tamarind Flesh As Anti-diabetes

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ABSTRACT

Indian borage leaves (*Coleus amboinicus*) at dose of 620mg/KgBB and tamarind (*Tamarindus indica*) at dose of 300mg/KgBB effectively decrease glucose contain in blood with work mechanism by reducing oxidative stress in body. Flavonoid contain in these plants was thought to play role as anti-diabetes. Because of the efficacy similarity of these plants, so the combination of Indian borage and tamarind extract was made in liquid herbal dosage form. The liquid herbal in this research were intended to oral use, so they can be given additional flavoring, sweetener, or water- soluble dyes to improve dosage quality. Liquid herbal dosage were made in 4 formulas based on different types of sweeteners, the test result showed that liquid herbal with palm sugar sweetener was the most preferred by panellists. Selected formulas from the test of preference was tested for quality testing include : stabilization test at three different temperatures over a period of 8 weeks with parameters pH measuring, viscosity, specific gravity, organoleptic, determination of flavonoid content and testing of microbial contamination. Stability test result showed a change in the quality and decrease in flavonoid levels in liquid herbals dosage every week. Based on data on the decrease in flavonoid contain in stability test, the prediction of the shelf life of liquid herbals dosage was obtained for 8 week at room temperature.

Keywords: Liquid herbal, Indian borage leaves, Tamarind, Antidiabetes, Stability test

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INTRODUCTION

Diabetes mellitus is a disease characterized by high levels of glucose in the blood due to disturbances in insulin secretion, insulin sensitivity or both which cause disruption carbohydrate, protein, and fat metabolism (Dipro, 2015). The number of diabetics every years is always increasing, this can be seen from the IDF (International Diabetes Federation) data. Outsurgova (2017) conducted a case study on diabetes, the result of the study stated that in 20140 diabetics in the world are predicted to experience an increase of 10.4% with the number of sufferers reaching 642 million people.

To reduce the number of diabetics who continue to increase each year can be done the development and utilization of plants that have the potential as antidiabetic, such as Indian borage (*Coleus amboinicus*) and tamarind (*Tamarindus indica*). Based on the results of the preclinical test conducted by Suryowati (2015) the thick extract of Indian borage leaves at a dose of 620mg/kg BB effectively reduces glucose levels in the blood. While the preclinical test results of dried tamarind flesh was made in liquid herbal dosage forms with the aim of increasing the effectiveness of the content of flavonoids which are thought to act as antidiabetic.

Consideration in choosing liquid herbal dosage forms due to faster absorption on the body when compared to other dosage forms, besides that dose of the extract as antidiabetic is large enough so that it is not possible to make it in tablet dosage forms. To cover up bad odors and taste in liquid herbals, a variety of sweeteners with a low glycaemic index is added and safe for diabetics such as honey, stevia, sucralose and palm sugar.

Research Method

This research was conducted in March to June 2019 at the pharmacy Laboratory of the Faculty of Mathematics and Sciences Pakuan University Bogor.

Research Tools

The tools used were sieve 40 (Amb[®]) mesh, oven (Memert[®], Schwabach, Germany), autoclaves (E-Scientific[®], Indianapolis, USA), and vacuum dry (Ogawat[®], Ogawa, Japan). The tools used for the analysis were glass, currency, refrigerator, picnometer, pH meter (Ohaus[®], Newark, New Jersey), spectrophotometer UV-Vis V-730 (Jasco[®], Amityville, New York), centrifugation (Hettich[®], Beverly, United States), furnace (Ney[®], United States), analytic scale (And[®]), and viscometer (Brookfield DV-II+ Pro[®]).

Research Materials

Raw materials used were Indian borage obtained from Cikarawang-Bogor Mitra Wain Group plantations and tamarind flesh obtained from the Cimanggu-Bogor Spice and Medical Crops Research Institute (BALITTRO), ethanol 96% (Merck[®]), honey (Al-Shifa[®]), palm sugar, stevia (Stevia Truly Sweetener[®]), sucralose (JK Sucralose inc[®]), aquades, apples essence (Brataco[®]), glycerine (Merck[®]), and natrium benzoat (Merck[®]). Material used for the analysis were Methanol, Natrium acetate, Aluminium Trychloride, Quercetin (Sigma-Aldrich, St. Louis, United states), Nutrient agar (Merck[®]), Buffered Pepton Water 0,1% (Merck[®]).

Research Method

The making of Simplicia Powder of Indian Borage leaves and Tamarind Flesh

The stage of simplification are started from raw material collection, plant determination, wet sorting, washing, drying and dry sorting. The results of simplicia are pollinated then sieved with 40 mesh sieve.

The making of Dry Extract of Indian Borage Leaves and Tamarind Flesh

Simplicia powder of Indian borage was extracted by maceration method by soaking the simplicia to ethanol 96% at room temperature (Hanai, 2015). Simplicia powder of tamarind flesh was extracted by infusion method by using water solvent, at 96 -98°C for 15-20 minutes (Hanai,2015). Liquid extract obtained from the extraction process dried by using dry vacuum to vanishing the solvent, then dried extract was obtained.

Dry Extract Characterization

Dry extract characterization include phytochemical analysis in accordance with procedure in *Materia Medika Indonesia* volume IV (1995), determination of water contain by gravimetric method and determination of ash contain extract.

Determination of Flavonoid Contain

Determination of flavonoid contain was done to extract and dosage liquid herbal. Determination of flavonoid contain was done based on chang method.

The Making of Comparative Standard solution

Standard quercetin weighed as much as 100mg, dissolved in 100 mL of methanol (1000 ppm). Then the solution was piped as much as 10 mL (100 ppm). Then Standard solution of quercetin with concentration 100 ppm was made solution concentration series as much as 2, 4, 6, 8, and 10 ppm by pipetting Standard solution of quercetin 100 ppm as much as 1, 2, 3, 4, and 5 mL from solution quercetin 100 pm then put into a flask measure 100 mL. Standard solution of quercetin was each added with 1 mL AlCl₃ 10%, 1 mL natrium acetate 1M and added distilled water until

the boundary mark. Then incubated for 30 minutes at room temperature. The absorbance of the liquid was measured by spectrophotometer UV-Vis at maximum wavelength of 431 nm.

The Making of Solution Sample

The solution sample was made by weighing dry extract of Indian borage as much as 0,6 g and dry extract of tamarind flesh weighing as much as 1 gram then the extracts were mixed and put into a 50 mL volumetric flask and added methanol to the boundary mark. Then the solution was piped as much as 2 mL and added with 1 mL AlCl₃ 10%, 1 mL natrium acetat 1M and added distilled water until 50 mL. The sample was incubated at the optimum time, then the solution was measured for absorption at maximum wavelength. For the analysis of the flavonoid contain in liquid herbal had the same procedure. Determination of total flavonoid contain stated as quercetin.

The Formulation of Liquid Herbal of Mixed India Borage Leaves Extract and Tamarind Flesh Extract

Liquid herbal dosage made in 4 formula based on the different sweeteners used, each formula of liquid herbal made as much as 250 mL. The rules for using liquid herbal was by drink it once as much as 25 mL for adults. Each 25 mL liquid herbal contain 0,6 g dry extract of indian borage and 1 g dry extract of tamarind leaves. Formulation of additional Substances can be see in table 1.

Table 1. Formulation of Additional Substances

Additional Substances	Formulation (%)			
	F1	F2	F3	F4
Honey	18	-	-	-
Sucralose	-	0,45	-	-
Stevia	-	-	0,60	-
Palm Sugar	-	-	-	25
Glycerin	10	10	10	10
Sodium benzoate	0,28	0,28	0,28	0,28
Apple Flavouring	0,01	0,01	0,01	0,01
Aquadest ad	100	100	100	100

The making of liquid herbal by extracting tamarind flesh dissolved in hot distilled water as much as 50 mL then filtered (solution 1). Indian boarage leaves extract dissolved into ethanol then stirred using homogenizer until the extract dissolved, then glycerine and distilled water until dissolved then filtered (solution 3). Natrium benzoate dissolved in hot distilled water as much as 20 mL of hot distilled water until dissolved then filtered (solution 4).

Solution 3 was inserted into the beaker glass, then added solution 1, 2, and 4 were stirred using homogenizer until homogeneous. Then the essence of the apple which had been dissolved with hot

distilled water as sufficient. The mixture was added distilled water to the 250 mL mark. Liquid herbal dosage was then cooled and then put into a sterilized brown glass bottle container.

Evaluation Liquid herbal Dosage

Preference Test of Liquid herbal

Preference test was carried by 20 panellists age over 30 years. Preference test parameters include aroma, color, and liquid herbal taste.

Stability Test of Liquid Herbal

The liquid herbal stability test carried out on the formula which preferred the results of the preference test. A total of 10 liquid herbal bottles placed in cool temperature (5-15°C), room (25-30°C), and accelerated temperature (40-45°C) then the liquid herbal stored for 8 weeks and at certain times were observed the parameters test include organoleptic, pH, specific gravity, viscosity, clarity test and determination of flavonoid content.

Microbial test of Liquid Herbal

Test of microbial contamination was carried out to determine the presence of microbial contamination in liquid herbal dosage by means of a total plate number test. Calculated the number of bacterial colonies in serial dilution then results obtained were compared with the standard, requirement for syrup was $< 1 \times 10^4$ colony/ml (SNI 7388:2009).

RESULTS AND DISCUSSION

Each formula of liquid herbal had sweet flavour, brown with apple aroma. The result of quality evaluation of each formula of liquid herbal showed different result, it was affected by different sweeteners used. Liquid herbal dosage based on the preference test the result was formula 3 with palm sugar sweetener became the most preferred by the panellist in terms of aroma, colour, and taste. Stability test liquid herbal formula 3 showed changes in quality and decrease in flavonoid levels in the dosage. Based on the data on the decrease in flavonoid levels in the dosage, the age calculation results of storing stable liquid herbal dosage were stored at room temperature for 8 weeks.

The result of dry extract showed that the result of phytochemical test on Indian borage contains flavonoid, tannin, and terpenoid. Tamarind extract contains alkaloid, flavonoid, terpenoid, glycoside and saponin. The data of water contain, ash contain, and flavonoid contain of the extract can be seen in Table 2.

Table 2: The result of water contain, ash contain, and flavonoid contain on the extract

	Water contain	Ash contain	Flavonoid contain
Dry extract of Indian borage	6,1685±0,0620	4,6933±0,0196	1,4283±0,0109
Dry extract of Tamarind	8,4904±0,1170	4,1612±0,0243	0,3252±0,0017
Combination	7,2307±0,3270	7,5562±0,1751	0,7456±0,0033

Herbal preparation are made based on different types of sweeteners, formula 1 (stevia), formula 2 (honey), formula 3 (palm sugar) and formula 4 (sucralose) and then evaluate the quality of formula. The organoleptic test shows all liquid herbal formulas are brown, sweet with an apple aroma.

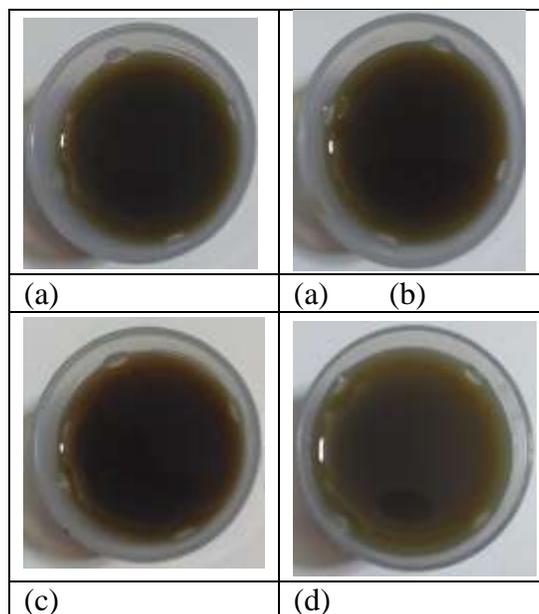


Figure 1: Liquid herbal mixed of Indian borage extract and tamarind flesh with sweeteners (a) stevia; (b) honey; (c) palm sugar; (d) sucralose

The result of pH measurement, specific gravity and viscosity had differences on every formula can be seen in Table 2. The differences were affected by different sweeteners used.

The result of liquid herbal preferences test showed formula 3 with palm sugar sweetener was preferred by the panellists in terms of taste, aroma, and color. Formula 3 with palm sugar sweetener was carried out stability test to know the quality changes happened during storage and to predict the shelf life. Stability test showed changes in organoleptic, pH, viscosity, specific gravity, and deposits formed in the liquid herbal dosage in every week, it was affected by temperature and enzymatic process during storage. The test results of microbial contamination with the total plate number method at week-0 and week-8 did not meet the requirements because the formation of colonies in serial dilution of liquid herbal dosage, microbial contamination formed was thought to be to lack of preservative concentration in formulation of liquid herbal dosage. Flavonoids levels

in liquid herbal dosage decreased every week. Graphic of Decreasing Flavonoid levels can see on table picture 2.

The results of the flavonoid levels of herbal liquid dosage decreased due to the levels of flavonoid contained in the extract stored in the form of liquid herbal dosage so that the temperature and duration of storage can affect the levels of flavonoid. Based on data on the decrease in flavonoid levels in the dosage resulted the calculation of shelf life of liquid herbal would be stable stored at room temperature for 8 weeks.

Table 3: The Result of Quality Evaluation of the Liquid Herbal Dosage

Formula	Parameter Uji		
	Viskositas (Cps)	Bobot Jenis (g/mL)	pH
1	25,0±0,2	1,0439±0,00150	3,531±0,0027
2	26,0±0,0	1,0449±0,00030	3,493±0,0075
3	25,0±0,2	1,0523±0,00005	3,706±0,0020
4	23,4±0,2	1,0363±0,00040	3,525±0,0045

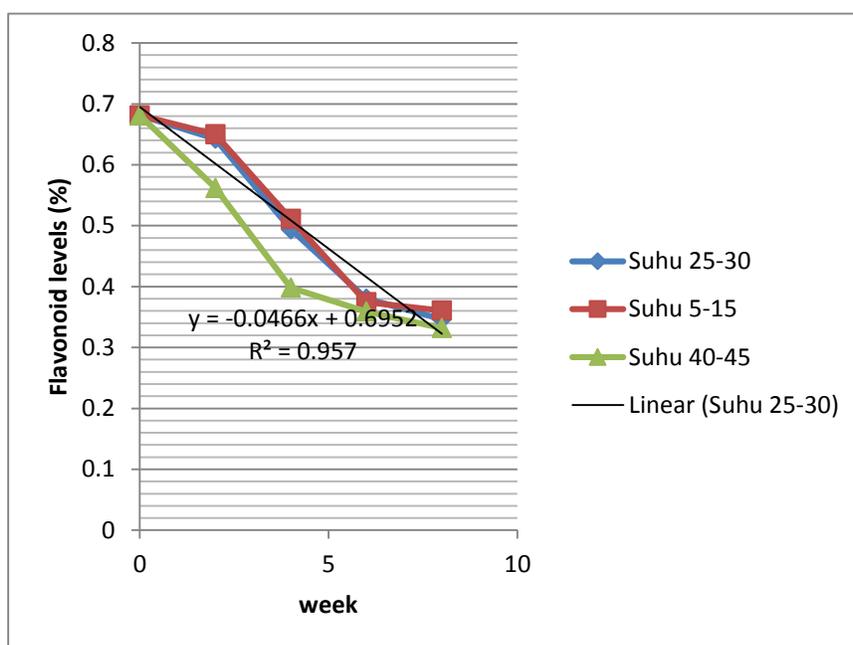


Figure 2: Graphic of Decreasing Flavonoid levels

CONCLUSION

Herbal liquid mixed of Indian borage and tamarind flesh in formula 3 with palm sugar as the sweetener had the most preferred color, taste, and aroma by the panellist Based on the stability test the changes of the quality of the liquid herbal dosage happened every week in all temperature. Flavonoid contain in liquid herbs dosage decreased during the period of storage and predicted shelf life of liquid herbal dosage for 8 weeks in room temperature

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