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DEVELOPMENT OF SOURSOP FRUIT INSTANT GRANULES (*ANNONA MURICATA* LINN) FROM FRUIT JUICE, ETHYL ACETATE AND ETHANOL EXTRACT AS LOWERING URIC ACID AND BLOOD PRESSURE

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Key words : *Instant granules, Juice, Ethanol extract and ethyl acetate extract, Soursop fruit (Annona muricata Linn)*

Abstract - Development of soursop fruit as uric acid- lowering and blood pressure has not been done. Soursop contains vitamin C, polyphenols, Na and K, which used in the treatment of degenerative diseases. The purpose of this study is to produce a healthy drink as instant granules which have efficacy in lowering uric acid and blood pressure. The extract used was a dry extract from fruit juice, ethyl acetate and ethanol extract. The results of the characterization of dry extract such as moisture content and ash content <10%, it means meet the requirements. The toxicity tests showed that LC_{50} of fruit juice powder <dry extract ethanol < dry extract ethyl acetate. The preparation consists of 3 (three) formulas instant granules. Instant granules prepared by wet granulation method. The results showed granule instant formulas from fruit juice and ethanol extract has a flow rate and angle of response easy flow, while the ethyl acetate extract of difficult flowing. All of instant granules have a sour taste sweet and specific odour. Time dispersion throughout the granules <5 minutes. A test based on formula showed that most panelists preffered juice (F1) ethyl acetate extract (F3) and ethanol extract (F1). The analysis content of vitamin C, polyphenols, Na and K in the dry extract is greater than granules. The compounds content of fruit juice more than ethanol and ethyl acetate extract. All of these results indicate instant granules with a quite good quality is obtained from fruit juice and ethanol extracts.

INTRODUCTION

Soursop plant has long been used as traditional medicine, many attention from the public because of the news from the media regarding the efficacy in killing cancer cells. Soursop leaf oil is known to have in vitro cytotoxic activity against cancer cells (Owolabi *et al*., 2013) while the ethanol extract of the leaves as a potent antibacterial against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Vijayameena *et al.*, 2013). In Indonesia, soursop grow well in areas that have a height of less than 1000 meters above sea level (Thomas, 1992). The fruit contains a lot of carbohydrates, especially fructose. The content of vitamin C, vitamin B1 and B2 are also quite high (Astawan, 2011). According Pellsser *et al*., (1994) on leaves and fruit identified 59 components, especially β-kariopylen 31.4%, o-kadinen 6.7%, 5.5% α-murolen, τ and a kadinol 4.3%. According to Cosmo *et al*., (2007) on β-kariopylen leaves contain 40% and the seeds contain 25% o-phelandren.

Soursop leaves can be used as anticonvulsant and to cure ulcers (Thomas, 1992). Extracts of the leaves and stems made by Durand *et al*., (1962) given by injection, have the temporary depressor effect on blood pressure. Empirically soursop fruit has been used to lower uric acid and reduce hypertension (Taylor, 2002). Uric acid-lowering activity as related to the content of vitamin C and polyphenol compounds that have antioxidant activity (Mardiana, 2012; Fianti, 2010). Activity soursop fruit as uric acid-lowering probably of antioxidant compounds that can inhibit the work through competitive inhibition of xanthine oxidase with xanthine substrate groups (Hidayat, 2007).

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Hypertension can be caused by several factors such as genetic factors, stress, obesity, life style such as eating foods with high cholesterol and consuming foods containing excessive salt (Mancia *et al.*, 2007) and the activity of soursop fruit as lowering hypertension is associated with low levels of sodium/ Na (14 mg/100 g) but high levels in potassium/ K (278 mg/l00 g). Comparison of potassium and sodium is beneficial in preventing hypertension (Purnomo, 2012). It is also stated by Waring *et al.*, (2005) and Hayden and Tyagi, (2004) that a high potassium intake will increase the concentration in the intracellular fluid, so it tends to attract the extracellular fluid and lowers blood pressure.

Bora *et al.*, (2004) reported that soursop fruit has a high polyphenol content. According to Astawan, (2011) compounds with high polyphenol content has a high antioxidant activity that could inhibit the enzyme xanthine oxidase. This enzyme catalyzes the conversion function for purines into uric acid so it can inhibited uric acid formation as well (Waring *et al.*, 2005 and Feig *et al.*, 2008). Soursop fruit is also reported to contain lots of fiber and speeds up urination due to high water content (Mardiana, 2012).

Characteristics of taste and odour of fresh soursop fruit are very suitable to serve beverage products such as instant granules. The advantage of granules preparation is practicability in use. Granule preparations is clumps of smaller particles, not uniform and become as single particles, mesh size ranges from 4-2, but can be made according to the wishes and purpose of use. Granulation is the process of converting the powder mixture into granules that are free flowing than the original powder (Ansel, 1989). The dosage form is focused on use as a health drink that maintains the continuity soursop fruit, considering that it is not durable. Utilization of soursop fruit in a variety of modern products is needed as diversification of products to be more easily consumed and the public interest as well as to increase the value-addition (Sari *et al.*, 2012). According to Luo *et al.*, (2012) 98.2% of research indicates that there is no difference statistically significant in effectiveness between the granule products and fresh herbal products. Additional materials which are used in the production of instant granules such as sucralose, polyvinil-pirolidon, NaCl and lactose. The aim of this study is to find a healthy drink in the form of instant granules which has efficacy in lowering uric acid and blood pressure so as to improve public health for the development of country.

MATERIALS AND METHODS

This research was conducted in the Laboratory of Pharmacy, Faculty of Mathematics and Natural Sciences Pakuan University, Bogor, Central Research Institute of Botany Field Biology-LIPI, Laboratory of Cattle Research-Ciawi, and the Laboratory of Bogor Agricultural University. The study began in February to June 2014, with the preparation of the fruit to be dried powder from juice, ethanol extract and ethyl acetate extract. Three types of dry powders were tested for toxicity with Brine Shrimp Lethality Test method / BSLT (Meyer), and then formula for instant granules was made. The focus of this research is in finding additional materials and appropriate methods in order to produce a good preparation with any formulas. The products will be tested for pharmaceutical parameters, consumer acceptance (hedonic test) and analysis of the content of vitamin C, polyphenols, Na and K in the granules.

Preparation of Powder from Soursop Fruit

Ripe soursop fruit 100 kg of which has been determined in LIPI, was separated from the skins, seeds and middle part the final yield was 69 kg. Then each 20 kg of it parts made into fruit juice, ethanol and ethyl acetate extracts. Fruit juice is made by squeezing the fresh fruit using flannel, while the manufacture of ethanol and ethyl acetate extract were prepared by maceration method. Fruit juice dried with freeze dryer previously added 20% maltodextrin. The ethanol extract was evaporated with a rotary evaporator, then dried by vacuum dryer plus 35% maltodextrin. The ethyl acetate extract dried by spray dryer plus 35% maltodextrin.

Toxicity Test of Powder

Toxicity tests conducted on fruit juice, ethanol and ethyl acetate extract powder with Brine Shrimp Lethality Test/BSLT using Meyer ways. This method uses the mortality indicator of *Artemia salina* L. shrimp larvae. The results are calculated as the value of LC_{50} (Lethal Concentration), the concentration of material that can cause death of shrimp larvae more than 50% after 24-hour incubation period. Test material with LC_{50} <1000

μg/mL can be considered as active or toxic compounds (Harmita, 2008).

A total of 100 μL of seawater containing 10-12 shrimp larvae were pipetted, inserted into the test container, added the solution to be tested with concentrations of 10, 100, 200, 500 and 1000 ppm and stirred, each concentration was repeated 3 times. A control was without the addition of the test material. The solution was left to stand for 24 hours, then counted the number of larvae which were dead and still alive from each container. Furthermore, mortality is calculated by means of the number of dead larvae divided by (total) multiplied by 100%. Graphs were made with the log concentration as the x-axis on mortality as the y-axis. LC_{50} value is the concentration of a substance that causes death of 50% is obtained by using the linear regression equation y = a + bx.

Qualitative Phytochemical Test

The powder of fruit juice, ethanol and ethyl acetate extract were tested for the content of flavonoids, alkaloids, tannins, saponins and polyphenols. Alkaloid content of qualitative test was performed with 3 types of reagents. Dragendroff's reagent (potassium bismuth nitrate), Mayer's reagent (potassium mercury iodide), and Wagner's reagent. Qualitative test of tannin done by 1% ferric chloride and gelatin test, saponins with soap test and hemolysis test (Rajendra *et al*., 2011). Polyphenols test with a solution of ferric chloride be 1%. All tests were repeated 3 times.

Quantitative Phytochemicals Test

Fruit juice powder was tested quantitatively for the content of polyphenols, vitamin C, Potassium and Sodium. The test was conducted using Prussian Blue (Gonzalez *et al*., 2003) and the determination of the amount of vitamin C conducted by iodometric titration method (Dioha *et al*., 2011). Test of Potassium and Sodium content was done in Cattle Research Institute, Ciawi-Bogor with Atomic Absorption Spectrophotometry method (Harmita, 2006). Quantitative test of polyphenols and vitamin C content was done in Laboratory Pharmacy with 3 replications.

Preparation of Instant Granules

Test results on the first stage showed that fruit juice, ethyl acetate and ethanol extract has potential as a uric acid-lowering and blood pressure lowering. The most effective dose in lowering uric acid levels and blood pressure is the basis for the manufacture of instant granules. At this stage some of the formulae will be made with different materials addition. Early experiment was carried out with many formulae that leads to the 3 formulae for each type of powder (Tables 1, 2 and 3), and each size of 20 g / sachet which was diluted with 200 mL of water. Method for making instant granules was dry granulation.

The resulting product will be tested pharmaceutical parameters, granule evaluation and hedonic test. Granule evaluation was conducted on the organoleptic, granule flow and angle of

Table 1. Formulae of Instant Granules Fruit Soursop Powder

Materials	Formula 1	Formula 2	Formula 3
Soursop fruit juice powder (%)	63	63	63
CMC (%)	1	1	1
Citric acid (%)	2	2	2
Sucralose (%)	0.1	-	0.035
Stevia (%)	-	1.2	0,075
Maltodextrin (%)	33.9	32.8	33.89

Table 2. Formulae of Instant Granules Ethyl Acetate extract powder

Materials	Formula 1	Formula 2	Formula 3
Ethyl Acetate Extract powder (%)	43.33	43.33	43.33
Binder (%)	PVP 2.5	-	CMC 1
Sucralose (%)	0.3	0.3	0.3
Tween (%)	1	1	1
Lactose (%)	ad 100	ad 100	ad 100

Table 3. Formulae of Instant Granules Ethanol Extract Powder

Materials	Formula 1	Formula 2	Formula 3
Ethanol extract powder (%)	66.67	66.67	66.67
Citric Acid (%)	2	2	2
Sucralose (%)	0,18	-	0,10
Stevia (%)	-	1	1
Lactose (%)	32.85	32.03	31.93

response test. Granule flow test carried out with 25 g of granules passed into the granule flow tester until the granules pass through a funnel, recorded time and measurements were performed 3 times. Calculation flow of granules was made using the formula: f = M / T, [f = rate flow (g / sec): T = time (seconds): M = weight granules (g)]. Determination of the type of granular flow based on statutes Aulton (1988) in Table 4.

Table 4. Type of Flow Based on the Value of Flowing Ability

Value of flowing ability (f)	Specification
>10	Free flowing
4 – 10	Easy flowing
1,4 – 4	Cohesive
<1,4	Highly cohesive

Determination of the angle of response is done by inserting a funnel into the granule mass. Falling mass will form a cone, and measured height and diameter of the cone, this test is done 3 times, in the determination of the angle of rest is done by the equation: Tan-1 a = hx / r and the determination of the type of flow based on the angle of rest can be seen in Table 5.

Table 5. Type of Flow Based on Angle of Response

Angle of response (a)	Specification
< 250	Very Easy Flowing
250<a<400	Easy Flowing
>400	Difficult Flowing

Solubility test is done with a sachet of instant granules was added to 200 mL of water, calculated the overall time required to instant granules dissolve / disperse. The hedonic test was conducted on 20 panelists with over 20 years of age. The panelists were asked to taste and make a score for the colour, taste, and odour of the sample. The panelists are expected to fill out a paper questionnaire that has been provided.

RESULTS AND DISCUSSION

Powder of Soursop Fruit

Powder of soursop fruit results are presented in Table 6, the highest yield of ethanol extract powder is 29.6%, compared to the ethyl acetate extract powder which is only 9.5%. It proves that ethanol can attract more active substances compared with ethyl acetate.

Toxicity Test of Powder

The results of toxicity tests with Brine Shrimp Lethality Test / BSLT showed the best values of LC50 powder from fruit is 100.6 ppm, followed ethanol extract 382.643 ppm and ethyl acetate extract 622.130 ppm. All powder have LC50 values <1000 µg /mL, it can be considered to have good activity toxicity (Harmita, 2008). Overall toxicity test results are presented in Table 7.

Qualitative Phytochemical Test

The Phytochemical Test showed that powder from fruit juice, ethanol and ethyl acetate extract contain alkaloids, flavonoids, saponins, tannins (Table 8).

Hedonic Test

Based on the responses taste test with 20 panelists at least 20 years of age of the three formulas for each type of instant granules. Panelists preffered formula 1 of fruit juice, formula 1 of ethanol extract and formula 3 of ethyl acetate extract. (Table 9).

Quality Test of Granules

The test results showed that the taste of instant granules is not always followed by good quality of granules, among the three types of granules only 1 granules meets the criteria. The granules have better organoleptic character when compared with the other, it has good flow rate (free flowing), angle of response (easy flowing), solubility time 1

Table 6. Characterization of Powder

Type of powder	Colour	Taste	Odour	Yield
Fruit juice	white	Sweet-sour	Soursop	19.5 %
Ethanol extract	Brownish	Sweet-sour	Less soursop	9.5 %
Ethyl Acetate extract	Slightly brown	Sweet-sour	Less soursop	29.6 %

Table 7. Results of Toxicity Test

Type of powder	Letal Concentration (LC50)
Fruit Juice	100.600 ppm
Ethanol Extract	382.643 ppm
Ethyl Acetate extract	622.130 ppm

Tabel 8. Results of Phytochemical Test

Phytochemical Test	Type of powder		
	Fruit juice	Ethyl acetate extract	Ethanol extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponin	+	+	+
Tannins	+	+	+
Water content	3.58%	4.40 %	1.5%

Table 9. Hedonic Test Results of Instant Granules Formulas

Ingredient	Fruit juice formula		
	Ethanol extract formula (F 1)(%)		Ethyl acetate formula (F 3)(%)
Powder of soursop	63	66.67	43.33
CMC	1	-	1
Citric acid	2	2	1
Sucralose	0.1	0.18	0.3
Maltodextrin	33.9	-	-
Tween	-	-	2
Lactose	-	31.15	52.4

Table 10. Results of Instant Granules Quality

Parameter tests	Fruit juice formula	Ethanol extract formula	Ethyl acetate formula
1. Organoleptic			
- Colour	White	Brownish	Brownish
- Taste	Sweet-sour	Sweet-sour	Sweet-sour
-Odour	Soursop	Soursop	Soursop
2. Flow rate (g/sec)	16	1.72	0.172
	Free flowing	Cohesive	Very cohesive
3. Angle of response (0)	29.07	28.36	41.35
	Easy flowing	Easy flowing	Difficult flowing
4.Soluble / dispersion time	1 min 54 sec	4 min 5 sec	1 min 1 sec

minutes 54 seconds (Table 10).

Analytical Content of Vitamin C, Polyphenols, Potassium and Sodium

The results analysis of vitamin C from powder and granule instant presented in Table 11. It appears that the pulverizing process in general decreases the amount of vitamin C except in ethanol extract powder. Similarly, in the process of making granules for all preparations may increase the amount of vitamin C. It has been an advantage conditions for instant granule products.

Table 11. Analysis of Vitamin C

Type of ingredient	Powder*	Instant granules*
Fruit Juice	46.93	191.98
Ethanol extract	103.51	103.45
Ethyl acetate extract	47.05	52.73

Note : * = mg / 100 g of ingredient

The results of analysis of polyphenol content showed that the process of making granules generally lowers polyphenol content and do so powder except ethanol extract (Table 12). This is presumably as ethanol can attract polyphenol from fruit.

Table 12. Analysis Content of Polyphenols

Type of ingredient	Powder*	Instant granules*
Fruit Juice	2.40	1.70
Ethanol extract	4.19	0.59
Ethyl Acetate extract	0.69	0.23

Note : * = mg SAG / g ingredient

The results of the analysis content of potassium (K) and sodium (Na) showed that the process of making granules are generally lower the content do so powder except ethanol extract (Table 13). Granule formation process as a whole maintains the ratio K / Na remains high.

CONCLUSION

From this study it can be concluded that:

1. Ripe Soursop fruit in the form of powder from fruit juice, ethanol and ethyl acetate extract can be made instant granules products.
2. The panelists preffered taste of formula 1 of instant granules from fruit juice and ethanol extract also formula 3 for ethyl acetate extract.
3. Vitamin C content is generally higher in instant granules compared than the extract and juice powder, otherwise the polyphenol content is lower in the instant granules than powder.
4. Instant granules process generally could make lowering Na and K content but the ratio K / Na remains high.

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Table 13. Analysis of Potassium and Sodium content

Type of ingredient	Kalium*		Natrium*		K/Na ratio	
	Powder	Granules	Powder	Granules	Powder	Granules
Fruit Juice	0.47	0.40	0.09	0.13	5.2	3.1
Ethanol extract	0.65	0.33	0.03	0.02	21	16.5
Ethyl Acetate extract	0.40	0.17	0.03	0.10	13	1.7

Note : * = g / 100 g ingredient

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01

LEMBAR
HASIL PENILAIAN SEJAWAT SEBIDANG ATAU *PEER REVIEW*
KARYA ILMIAH : JURNAL ILMIAH

Judul Jurnal Ilmiah (Artikel) : Development of Sourshop fruit instant granules (Annonna muricate Linn) form furit juice ethyl acetat and ethanol extract as lowering uric acid and blood pressure

Jumlah Penulis : 4 orang
Status Pengusul : Penulis Utama
Identitas Jurnal Ilmiah
a. Nama Jurnal : Asian Journal of microbiology dan environmental Sciences (AJM)
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Kategori Publikasi Jurnal Ilmiah :
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CATATAN PENILAIAN

- Abstrak sudah sesuai, terdiri dari tujuan, metoda & hasil yang diperoleh
- Ruang lingkup sudah memadai sesuai tujuan kegiatan
- Metodeji sudah sesuai dengan tujuan penelitian dan menggunakan acuan yang terupdate
- kualitas penerbit, sudah memenuhi syarat untuk jurnal internasional

Reviewer 1

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Total = (100%)	24			24

CATATAN PENILAIAN

Abstrak : Sudah sesuai dan menjelaskan Latar belakang, Tujuan dan metodologi serta Novelty dan hasil yg dijelaskan

Latar Belakang : Untuk mendukung Latar belakang sebagai dasar analisis sudah sesuai dan didukung oleh jurnal-jurnal bereputasi

Metodologi sudah sesuai setandar pembahasan Metodologi karya Ilmiah

Result & discuss : Analisa dan diskusi, sudah menjawab Tujuan dengan dukungan hasil analisis kuantity berupa dokumentasi Tabulasi, sehingga hasil analisi suatu) Komprehensif

Kesimpulan : sudah menjawab Hasil analisis dan diskusi

Reviewer 2

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Development of Soursop Fruit Instant Granules (Annona muricata Linn) from Fruit Juice, Ethyl Acetate and Ethanol Extract as Lowering Uric Acid and Blood Pressure

by Prasetyorini Unpak

Development of Soursop Fruit Instant Granules (*Annona muricata* Linn) from Fruit Juice, Ethyl Acetate and Ethanol Extract as Lowering Uric Acid and Blood Pressure

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ABSTRACT

Development of soursop fruit as uric acid-lowering and blood pressure has not been done. Soursop contains vitamin C, polyphenols, Na and K, which used in the treatment of degenerative diseases. The purpose of this study is to produce a healthy drink as instant granules which have efficacy lowering uric acid and blood pressure. The extract used was a dry extract from fruit juice, ethyl acetate and ethanol extract. The results of the characterization of dry extract such as moisture content and ash content <10%, it means meet the requirements. The toxicity tests showed that LC_{50} of fruit juice powder < dry extract ethanol < dry extract ethyl acetate. The preparation consists of 3 (three) formulas instant granules. Instant granules prepared by wet granulation method. The results showed granule instant formulas from fruit juice and ethanol extract has a flow rate and angle of response easy flow, while the ethyl acetate extract of difficult flowing. All of instant granules have a sour taste sweet and specific odour. Time dispersion throughout the granules < 5 minutes. A test based on formula showed that most panelists preffered juice (F1) ethyl acetate extract (F3) and ethanol extract (F1). The analysis content of vitamin C, polyphenols, Na and K in the dry extract is greater than granules. The compounds content of fruit juice more than ethanol and ethyl acetate extract. All of these results indicate instant granules with a quite good quality is obtained from fruit juice and ethanol extracts.

Keywords: instant granules, juice, ethanol extract and ethyl acetate extract, soursop fruit (*Annona muricata* Linn)

INTRODUCTION

Soursop plant has long been used as traditional medicine, many attention from the public because of the news from the media regarding the efficacy in killing cancer cells. Soursop leaf oil is known to have in vitro cytotoxic activity against cancer cells (Owolabi et.al, 2013) while the ethanol extract of the leaves as a potent antibacterial against Pseudomonas aeruginosa and Staphylococcus aureus (Vijayameena et al, 2013). In Indonesia, soursop grow well in areas that have a height of less than 1000 meters above sea level (Thomas, 1989). The fruit contains a lot of carbohydrates, especially fructose. The content of vitamin C, vitamin B1 and B2 are also quite a lot (Astawan, 2011). According Pellsser et al, 1994 on leaves and fruit identified 59 components, especially β-kariopylen 31.4%, o-kadinen 6.7%, 5.5% α-murolen, τ and α kadinol 4.3%. According to Cosmo et al, 2007 on β-kariopylen leaves contain 40% and the seeds contain 25% o-phelandren.

Soursop leaves can be used as anticonvulsant and to cure ulcers (Thomas, 1992). Extracts of the leaves and stems made by Durand et al, 1962 given by injection, have the temporary depressor effect on blood pressure. Empirically soursop fruit has been used to lower uric acid and reduce hypertension (Taylor, 2002). Uric acid-lowering activity as related to the content of vitamin C and polyphenol compounds that have antioxidant activity (Mardiana, 2012 and Fianti, 2010). Activity soursop fruit as uric acid-lowering probably of

antioxidant compounds that can inhibit the work through competitive inhibition of xanthine oxidase with xanthine substrate groups (Hidayat, 2007).

Hypertension can be caused by several factors such as genetic factors, stress, obesity, life style such as eating foods with high cholesterol and consuming foods containing excessive salt (Mancia *et al*, 2007), and the activity of soursop fruit as lowering hypertension associated with low levels of sodium/ Na (14 mg / 100 g) but high levels in potassium/ K (278 mg/l00g). Comparison of potassium and sodium has benefit for preventing hypertension (Purnomo, 2012). It is also stated by Waring et. al., (2005) and Hayden and Tyagi, (2004) that a high potassium intake will increase the concentration in the intracellular fluid, so it tends to attract the extracellular fluid and lowers blood pressure.

Bora *et al*, (2004) reported that soursop fruit has a high polyphenol content. According Astawan, (2011) compounds with high polyphenol content has a high antioxidant activity that could inhibit the enzyme xanthine oxidase. This enzyme catalyzes the conversion function for purines into uric acid so it can inhibited uric acid formation as well (Waring et al, 2005 and Feig *et a*l, 2008). Soursop fruit is also reported to contain lots of fiber and to speed up urination due to high water content (Mardiana, 2012).

Characteristics of taste and odour fresh soursop fruit very suitable to serve beverage products such as instant granules. The advantage of granules preparation than the other is practicability in use. Granule preparations is clumps of smaller particles, not uniform and become as single particles, mesh size ranges from 4-2, but can be made according to the wishes and purpose of use. Granulation is the process of converting the powder mixture into granules were free flowing than the original powder (Ansel, 1989). The dosage form is focused for use as a health drink that maintained continuity soursop fruit, considering not durable and limited. Utilization of soursop fruit in a variety of modern products needed as diversification of products to be more easily consumed and the public interest as well as to increase the value-added (Sari, *et al*, 2012). According to Luo *et al*, 98.2% of research indicates that there is no difference statistically significant in effectiveness between the granule products and fresh herbal products. Additional materials which used in the production of instant granules such as sucralose, polyvinylpirolidon, NaCl and lactose. The aim of this study is to find a healthy drink in the form of instant granules which has efficacy lowering uric acid and blood pressure so as to improve public health for the development of country.

MATERIALS AND METHODS

This research was conducted in the Laboratory of Pharmacy, Faculty of Mathematics and Natural Sciences Pakuan University, Bogor, Central Research Institute of Botany Field Biology-LIPI, Laboratory of Cattle Research-Ciawi, and the Laboratory of Bogor Agricultural University. The study began in February to June 2014, with the preparation of the fruit to be dried powder from juice, ethanol extract and ethyl acetate extract. Three types of dry powder tested toxicity with Brine Shrimp Lethality Test method / BSLT (Meyer), then they made formula as instant granules. The focus of this research is finding additional materials and appropriate methods in order to produce a good preparation with any formulas. The products will be tested pharmaceutical parameters, consumer acceptance (hedonic test) and analyse the content of vitamin C, polyphenols, Na and K in the granules.

Preparation of Powder from Soursop Fruit

Ripe soursop fruit as much as 100 kg of which has been determined in LIPI, separated from the skins, seeds and middle part, yield was 69 kg. Then each 20 kg of it parts made fruit juice, ethanol and ethyl acetate extracts. Fruit juice is made by squeezing the fresh fruit using flannel, while the manufacture of ethanol and ethyl acetate extract prepared by

maceration method. Fruit juice dried with freeze dryer previously added 20% maltodextrin. The ethanol extract evaporated with a rotary evaporator, then dried by vacuum dryer plus 35% maltodextrin. The ethyl acetate extract dried by spray dryer plus 35% maltodextrin.

Toxicity Test of Powder

Toxicity tests conducted on fruit juice, ethanol and ethyl acetate extract powder with Brine Shrimp Lethality Test / BSLT using Meyer ways. This method uses the mortality indicator of Artemia salina L. shrimp larvae. The results are calculated as the value of LC50 (Lethal Concentration), the concentration of material that can cause death of shrimp larvae more than 50% after 24-hour incubation period. Test material with LC50 <1000 ug / ml can be considered as active or toxic compounds (Harmita, 2008).

A total of 100 µL of seawater containing 10-12 shrimp larvae were pipetted, inserted into the test container, add the solution to be tested with concentrations of 10, 100, 200, 500 and 1000 ppm and stirred, each concentration was repeated 3 times. As a control carried out without the addition of the test material. The solution was left to stand for 24 hours, then counted the number of larvae were dead and still alive from each container. Furthermore, mortality is calculated by means of the number of dead larvae were reared larvae divided by (total) multiplied by 100%. Graphs were made with the log concentration as the x-axis on mortality as the y-axis. LC50 value is the concentration of a substance that causes death of 50% is obtained by using the linear regression equation $y = a + bx$.

Qualitative Phytochemical Test

The powder of fruit juice, ethanol and ethyl acetate extract tested for the content of flavonoids, alkaloids, tannins, saponins and polyphenols. Alkaloid content of qualitative test performed with 3 types of reagents that Dragendroff's reagent (potassium bismuth nitrate), Mayer's reagent (potassium mercury iodide), and Wagner's reagent. Qualitative test of tannin done by 1% ferric chloride and gelatin test, saponins with soap test and hemolysis test (Rajendra et al., 2011). Polyphenols test with a solution of ferric chloride be 1%. All tests repeated 3 times.

Quantitative Phytochemicals Test

Fruit juice powder tested quantitatively for the content of polyphenols, vitamin C, Potassium and Sodium. It test was conducted using Prussian Blue (Gonzalez et al., 2003) and the determination of the amount of vitamin C conducted by iodometric titration method (Dioha et al, 2011). Test of Potassium and Sodium content was done in Cattle Research Institute, Ciawi-Bogor with atomic absorption spectrophotometry method (Harmita, 2006). Quantitative test of polyphenols and vitamin C content was done in Laboratory Pharmacy with 3 replications.

Preparation of Instant Granules

Test results on the first stage showed that fruit juice, ethyl acetate and ethanol extract has potential as a uric acid-lowering and blood pressure lowering. The most effective dose in lowering uric acid levels and blood pressure is the basis for the manufacture of instant granules. At this stage some of the formulas will be made with different materials addition. Early experiments carried out many formulas that leads to the 3 formulas for each type of powder (Tables 1, 2 and 3), and each size of 20 g / sachet which diluted with 200 mL of water. Method for making instant granules made by dry granulation.

Table 1. Formula of Instant Granules Fruit Soursop Powder

Materials	Formula 1	Formula 2	Formula 3
Soursop fruit juice powder (%)	63	63	63
CMC (%)	1	1	1
Citric acid (%)	2	2	2
Sucralose (%)	0.1	-	0.035
Stevia (%)	-	1.2	0,075
Maltodextrin (%)	33.9	32.8	33.89

Table 2. Formula of Instant Granules Ethyl Acetate extract powder

Materials	Formula 1	Formula 2	Formula 3
Ethyl Acetate Extract powder (%)	43.33	43.33	43.33
Binder (%)	PVP 2.5	-	CMC 1
Sucralose (%)	0.3	0.3	0.3
Tween (%)	1	1	1
Lactose (%)	ad 100	ad 100	ad 100

Table 3. Formula of Instant Granules Ethanol Extract Powder

Materials	Formula 1	Formula 2	Formula 3
Ethanol extract powder (%)	66.67	66.67	66.67
Citric Acid (%)	2	2	2
Sucralose (%)	0,18	-	0,10
Stevia (%)	-	1	1
Lactose (%)	32.85	32.03	31.93

The resulting product will be tested pharmaceutical parameters, granule evaluation and hedonic test. Granule evaluation was conducted on the organoleptic, granule flow and angle of response test. Granule flow test carried out with 25 g of granules passed into the granule flow tester until the granules pass through a funnel, recorded time, and measurements were performed 3 times. Calculation flow of granules made using the formula: f = M / T, [f = rate flow (g / sec): T = time (seconds): M = weight granules (g)]. Determination of the type of granular flow based on statutes Aulton (1988) in Table 4.

Table 4. Type of Flow Based on the Value of Flowing Ability

Value of flowing ability (f)	Specification
>10	Free flowing
4 – 10	Easy flowing
1,4 – 4	Cohesive
<1,4	Highly cohesive

Determination of the angle of response is done by inserting a funnel into the granule mass. Falling mass will form a cone, and measured height and diameter of the cone, this test is done 3 times, in the determination of the angle of rest is done by the equation: Tan-1 α = hx / r and the determination of the type of flow based on the angle of rest can be seen in Table 5.

Table 5. Type of Flow Based on Angle of Response

Angle of response (α)	Specification
$< 25^0$	Very Easy Flowing
$25^0 < \alpha < 40^0$	Easy Flowing
$> 40^0$	Difficult Flowing

Solubility test is done with a sachet of instant granules was added to 200 ml of water, calculated the overall time required to instant granules dissolve / disperse. The hedonic test conducted on 20 panelists with over 20 years of age. The panelists were asked to taste and make a score for the colour, taste, and odour of the sample. The panelists are expected to fill out a paper questionnaire that has been provided.

RESULTS AND DISCUSSION

Powder of Soursop Fruit

Powder of soursop fruit results are presented in Table 6, the highest yield of ethanol extract powder is 29.6%, compared to the ethyl acetate extract powder which is only 9.5%. It proves that ethanol can attract more active substances compared with ethyl acetate.

Table 6. Characterization of Powder

Type of powder	Colour	Taste	Odour	Yield
Fruit juice	white	Sweet-sour	Soursop	19.5 %
Ethanol extract	Brownish	Sweet-sour	Less soursop	9.5 %
Ethyl Acetate extract	Slightly brown	Sweet-sour	Less soursop	29.6 %

Toxicity Test of Powder

The results of toxicity tests with Brine Shrimp Lethality Test / BSLT showed the best values of LC50 powder from fruit is 100.6 ppm, followed ethanol extract 382.643 ppm and ethyl acetate extract 622.130 ppm. All powder have LC50 values <1000 ug / ml, it can be considered to have good activity toxicity (Harmita, 2008). Overall toxicity test results are presented in Table 7.

Table 7. Results of Toxicity Test

Type of powder	*Letal Concentration* (LC_{50})
Fruit Juice	100.600 ppm
Ethanol Extract	382.643 ppm
Ethyl Acetate extract	622.130 ppm

Qualitative Phytochemical Test

The Phytochemical Test showed that powder from fruit juice, ethanol and ethyl acetate extract contain alkaloids, flavonoids, saponins, tannins (Table 8).

Tabel 8. Results of Phytochemical Test

Phytochemical Test	Type of powder		
	Fruit juice	Ethyl acetate extract	Ethanol extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponin	+	+	+
Tannins	+	+	+

Water content	3.58%	4.40 %	1.5%

Hedonic Test

Based on the responses taste test with 20 panelists at least 20 years of age of the three formulas for each type of instant granules. Panelists preffered formula 1 of fruit juice, formula 1 of ethanol extract and formula 3 of ethyl acetate extract. (Table 9).

Table 9. Hedonic Test Results of Instant Granules Formulas

Ingredient	Fruit juice formula (F 1)(%)	Ethanol extract formula (F 1)(%)	Ethyl acetate formula (F 3)(%)
Powder of soursop	63	66.67	43.33
CMC	1	-	1
Citric acid	2	2	1
Sucralose	0.1	0.18	0.3
Maltodextrin	33.9	-	-
Tween	-	-	2
Lactose	-	31.15	52.4

Quality Test of Granules

The test results showed that the taste of instant granules is not always followed by good quality of granules, among the three types of granules only 1 granules that meet the criteria. The granules have better organoleptic character when compared with the other, it has good flow rate (free flowing), angle of response (easy flowing), solubility time 1 minutes 54 seconds (Table 10).

Table 10. Results of Instant Granules Quality

Parameter tests	Fruit juice formula	Ethanol extract formula	Ethyl acetate formula
1. Organoleptic			
- Colour	White	Brownish	Brownish
- Taste	Sweet-sour	Sweet-sour	Sweet-sour
-Odour	Soursop	Soursop	Soursop
2. Flow rate (g/sec)	16	1.72	0.172
	Free flowing	Cohesive	Very cohesive
3. Angle of response (^{0})	29.07	28.36	41.35
	Easy flowing	Easy flowing	Difficult flowing
4. Soluble / dispersion time	1 min 54 sec	4 min 5 sec	1 min 1 sec

Analytical Content of Vitamin C, Polyphenols, Potassium and Sodium

The results analysis of vitamin C from powder and granule instant presented in Table 11. It appears that the pulverizing process in general decrease the amount of vitamin C except in ethanol extract powder. Similarly, in the process of making granules for all preparations may increase the amount of vitamin C. It has been an advantage conditions for instant granule products.

Table 11. Analysis Content of Vitamin C

Type of ingredient	Powder*	Instant granules*
Fruit Juice	46.93	191.98
Ethanol extract	103.51	103.45
Ethyl acetate extract	47.05	52.73

Note : * = mg / 100 g of ingredient

The results analysis of polyphenol content showed that the process of making granules generally lowering polyphenol content and do so powder except ethanol extract (Table 12). This is presumably due to the ethanol can attract polyphenol from fruit.

Table 12. Analysis Content of Polyphenols

Type of ingredient	Powder*	Instant granules*
Fruit Juice	2.40	1.70
Ethanol extract	4.19	0.59
Ethyl Acetate extract	0.69	0.23

Note : * = mg SAG / g ingredient

The results of the analysis content of potassium (K) and sodium (Na) showed that the process of making granules are generally lower the content do so powder except ethanol extract (Table 13). Granule formation process as a whole maintains the ratio K / Na remains high.

Table 13. Analysis Content of Potassium and Sodium

Type of ingredient	Kalium*		Natrium*		K/Na ratio	
	Powder	Granules	Powder	Granules	Powder	Granules
Fruit Juice	0.47	0.40	0.09	0.13	5.2	3.1
Ethanol extract	0.65	0.33	0.03	0.02	21	16.5
Ethyl Acetate extract	0.40	0.17	0.03	0.10	13	1.7

Note : * = g / 100 g ingredient

CONCLUSION

From this study it can be concluded that:

1. Ripe Soursop fruit in the form of powder from fruit juice, ethanol and ethyl acetate extract can be made instant granules products.
2. The panelists preffered taste of formula 1 of instant granules from fruit juice and ethanol extract also formula 3 for ethyl acetate extract.
3. Vitamin C content is generally higher in instant granules compared than the extract and juice powder, otherwise the polyphenol content lower in the instant granules than powder.
4. Instant granules process generally could make lowering Na and K content but the ratio K / Na remains high.

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